PB83-116913

Effects of 200, 591 and 2450 MHz Microwaves on Cerebral Energy Metabolism

Duke Univ. Medical Center
Durham, NC

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

Oct 82

U.S. Department of Commerce
National Technical Information Service
NTIS

PB83-116913

EPA-600/1-82-014
October 1982

EFFECTS OF 200, 591 AND 2450 MHz MICROWAVES ON CEREBRAL ENERGY METABOLISM

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E.P.A. Contract Number 68-02-3233

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/1-82-014	2.
3. RECIPIENT'S ACCESSION NO. PB83-116913	
4. TITLE AND SUBTITLE Effects of 200, 591 and 2450 MHz Microwaves on Cerebral Energy Metabolism.	5. REPORT DATE October 1982 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Aaron P. Sanders and William T. Joines	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Duke University Medical Center Durham, NC 27710	10. PROGRAM ELEMENT NO. F104 11. CONTRACT/GRANT NO. 68-02-3233
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE
15. SUPPLEMENTARY NOTES	

16. ABSTRACT

Earlier work has shown that levels of key biochemicals in the energy production system of rat brain are affected by exposure to 591 MHz microwave radiation at 13.8 mW/cm^2. The objectives of this study were to determine whether there are direct microwave effects on the biological system or whether they are secondary to the hyperthermia produced in the tissue, to establish dose-response relationships for the effects, and to investigate different frequencies of exposure and modulation of the carrier signal. The fluorescence of reduced nicotinamide adenine dinucleotide (NADH) in the rat brain was measured in vivo during exposure to the microwave radiation, and adenosine triphosphate (ATP) and creatine phospate (CP) levels were measured chemically after exposure. An increase in brain temperature from 35.6 to 39^{0}C caused no change in NADH fluorescence, an 11.8% reduction in the ATP level and a 28.8% decrease in the CP level. Microwave exposures at 200 and 591 MHz for 0.5 to 5 minutes at 13.8 mW/cm^2 caused no measurable increase in brain temperature; however, the maximum NADH fluorescence increase was 10% while ATP levels decreased by as much as 30%. CP levels decreased by up to 40% at 591 MHz but not at all at 200 MHz. Similar exposures at 2450 MHz produced no changes in any of the parameters measured. These results indicate frequency specific inhibition of brain energy metabolism. At 200 MHz the mitochondrial NADH-to-ATP production pathway was inhibited. At 591 MHz both the NADH-to-ATP and the CP-to-ATP pathways were inhibited. At 2450 MHz, neither pathway was affected. The data support the hypothesis that microwave radiation directly inhibits mitochondrial energy production pathways in rat brain, and that tissue heating is not a factor.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT RELEASE TO THE PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 72
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

Form 2220-1 (9-73)

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FOREWARD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, nonionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for nonionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

This study, conducted by Duke University Medical Center, is intended to extend a preliminary research finding that nonionizing radiation affects energy metabolism in animal brain. A finding of this nature is important in forming the data base upon which a population standard for nonionizing radiation would be based. This report describes the results of experiments that define several parameters characterizing this effect.

F. G. Hueter, Ph.D.
Director
Health Effects Research Laboratory

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ABSTRACT

Previous work in this laboratory has shown that levels of key biochemicals in the energy production system of rat brain are affected by exposure to 591 MHz microwave radiation at 13.8 mW/cm^2. The objectives of this study were to determine whether the microwave effects on biological systems are direct or secondary to the hyperthermia produced in the tissue, to establish dose - response relationships for the effects and to investigate different frequencies of exposure and modulation of the carrier signal. Fluorometry was used to measure the inhibition of energy metabolism after exposing rat brain to microwave power densities, some of which are probably incapable of producing hyperthermia. The study included microwave exposures with brain surface power densities varying from 0.1 to 60 mW/cm^2 at 200, 591, and 2450 MHz, continuous wave (CW) propagation. The effects of exposure to sinusoidally modulated (4-32 Hz) and pulse modulated (5 μsec/pulse, 500 or 250 pps) microwaves were also investigated using a 591 MHz carrier at an average exposure intensity of 13.8 mW/cm^2.

Male Sprague Dawley rats (175-225 g) were anesthetized with urethane, scalp and muscle removed from the top and side of the skull and a 5 x 5 mm aperture was made through the skull leaving the dura intact. This permitted the use of fluorescence techniques for continuously recording brain NADH fluorescence. The animal with head in holder was positioned under a 3.75 cm diameter hole in the top plate of the microstrip exposure system. The excitation light was focused on the brain surface and the fiber optic bundle end was positioned approximately 45° to the excitation light beam and 1.5 to 2 cm from the excitation focal spot on the cerebral cortex. The fiber optics were divided 15 cm above the tip and each branch was connected to an optical filter-photomultiplier amplifier assembly (460 ± 5 nm and 549 ± 2 nm) then to recorder for continous recording of NADH fluorescence before, during and after microwave exposure. Animals for ATP and CP assay were located in the same position in the stripline without the head holder to permit more rapid quenching of brain ATPase and CPkinase when immersed in liquid N_2.

Increasing brain temperature from 35.6 to 39°C caused decreases in the concentrations of adenosine triphosphate (ATP) and creatine phosphate (CP) of 11.8 and 28.8% respectively. Microwave exposures (591 MHz, CW, 13.8 mW/cm^2) of 0.5 to 5 min caused no increase in brain temperature, but resulted in significantly decreased concentrations of ATP and CP in the 35.6 and 39°C rat brains. The 0.5 min exposure of the 35.6°C brain resulted in a 25.2% decrease of ATP when CP was at a level (60.6% of control) which usually sustains normal ATP concentration. The ATP and CP analyses of brain from 200 and 2450 MHz microwave exposure rats showed different effects than those animals exposed to 591 MHz.

Comparison of CW with 16 Hz sinusoidal amplitude modulation and 5 μsec

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500 pps pulsed exposures showed no significant difference in effects on brain NADH fluorescence or on concentration of ATP and CP. Data support the hypothesis that microwaves directly inhibit mitochondrial energy production, and this inhibition is a function of frequency and electric field strength in tissue.

This report was submitted in fulfillment of Contract No. 68-02-3233 under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period June 14, 1979 to October 14, 1980.

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ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

ADP	-- adenosine-5'-diphosphate
ATP	-- adenosine-5'-triphosphate
[ATP]	-- adenosine-5'-triphosphate concentration
C	-- celsius
cm	-- centimeter
CP	-- creatine phosphate
[CP]	-- creatine phosphate concentration
CP-kinase	-- creatine phosphokinase
CW	-- continuous wave
EMF	-- electromagnetic field
g	-- gram
Gz	-- gigahertz
HBW	-- half band width
Hz	-- hertz
IP	-- intraperitoneal
Kg	-- kilogram
MHz	-- megahertz
mg	-- milligram
mW	-- milliwatt
NAD	-- nicotinamide adenine dinucleotide
NADH	-- reduced nicotinamide adenine dinucleotide
nm	-- nanometer
P	-- probability
P_a	-- total absorbed power
P_{as}	-- stripline absorbed power
P_f	-- forward power
P_i	-- incident power
P_r	-- reflected power
P_t	-- transmitted power
pps	-- pulses per second
SAR	-- specific absorption rate
SEM	-- standard error of the mean
TEM	-- transverse electromagnetic
W	-- watt(s)

SYMBOLS

c	-- specific heat of tissue in calories per gram per °C
ε^*_r	-- relative complex permittivity of the tissue
δ	-- depth of penetration
f	-- frequency
V	-- velocity of light
κ	-- 4.186 joules/cal.
η	-- $\frac{\eta_o}{\sqrt{\varepsilon^*_r}}$ $\eta_o = 377$ ohms.
ρ	-- reflection coefficient
d	-- tissue density in grams per cc.
σ	-- conductivity of tissue
ΔT	-- temperature change in °C
τ	-- time in seconds
μ	-- micro
λ	-- wave length
z	-- depth in tissue in cm.

ACKNOWLEDGEMENTS

The open discussions of the controversial subjects in this field with Drs. John W. Allis and Joe A. Elder of The Cellular Biophysics Branch, Health Effects Research Laboratory, Environmental Protection Agency, Research Triangle Park, N.C., must be recognized as a direct contribution to the work in this report. The valuable technical assistance of Marvin Nunn, Julia Nunn, and Mark Rafal was significant to completion of this work.

SECTION I

INTRODUCTION

The question of whether there are biological effects of microwave exposure that are independent of tissue hyperthermia has been a matter of controversy for some time [1-7]. In addition, reports in the literature have shown that in some instances pulsed or sinusoidally modulated microwave exposures caused specific biological responses that were not observed with continuous wave (CW) microwave exposures [2,5,7,8]. Other reports have shown that biological effects from microwaves at specific levels of surface power density, or at specific absorption rates, disappeared at densities or rates both higher and lower: these critical ranges associated with biochemical changes have been called "power windows" [9,10]. In the light of these findings, all future study of microwave effects on biological system(s) should consider the following exposure conditions:

1. pulsed or sinusoidally amplitude modulated microwave exposures as well as continuous wave exposure;
2. power levels from 0.1 - 60 mW/cm^2 surface power density i.e., power levels below and including those known to produce general tissue thermogenesis some of which are below the present ANSI Recommended Safety Exposure Levels [11].
3. microwave frequencies that are significantly different to ascertain if the biological response is a general microwave effect or if it is frequency (or energy) dependent.

Earlier work in our laboratory tested the hypothesis that microwave exposures of tissue *in vivo* induce rotation or vibration in divalent ions or other functional groups in the electron transport chain, resulting in inhibition of the respiratory chain function followed by decreased adenosine triphosphate (ATP) and creatine phosphate (CP) concentrations [12].

The first entrance point to the electron transport chain, where nicotinamide adenine dinucleotide (NAD) is reduced to NADH, can be continuously monitored by excitation at 366 nm, and NADH fluorescence can be observed at 460 nm using a Chance time-sharing fluorimeter. NADH excitation-fluorimetry can be used to record continuously the relative NADH levels *in situ*. If there are stresses imposed upon the cells by microwaves that result in inhibition of respiratory chain function or inhibition of cell functions utilizing ATP, there would be an increase in NADH levels (and the fluorescence signal). Conversely, microwave enhancement of flow down the respiratory chain, or increased cellular functions that utilize energy (ATP) would result in a decrease in NADH levels and fluorescence. Thus, any microwave stress on cell functions, inhibition, or enhancement would be rapidly recorded by the change in the NADH fluorescence signal.

If microwaves impose a stress on mitochondria in the inhibition or enhancement of respiratory chain function, this stress will result in changes in concentrations of high-energy phosphate storage compounds, ATP and CP, in addition to changes in the redox state of NADH. However, if a suspension of isolated mitochondria were to be studied for possible microwave-induced changes in the redox state of NADH, it would have to be stirred to assure uniform oxygen and substrate concentrations. Stirring a mitochondrial suspension during microwave exposure would minimize or eliminate the possibility of detecting changes in the NADH redox state if the effect is dependent on the orientation of the electric or magnetic field. Thus, it was considered important that the initial microwave studies be performed <u>in vivo</u> so that electric-field orientation could be specified.

In the maintenance of ATP levels in the brain and heart, CP functions as a rapid back-up system in sustaining normal ATP concentrations. Brain ATP level is a function of the rates of ATP production and utilization and the rates of the rephosphorylation of ADP to ATP by the CP - CP-kinase reaction and mitochondrial respiration chain production of ATP. Schematically this is shown in Figure 1.

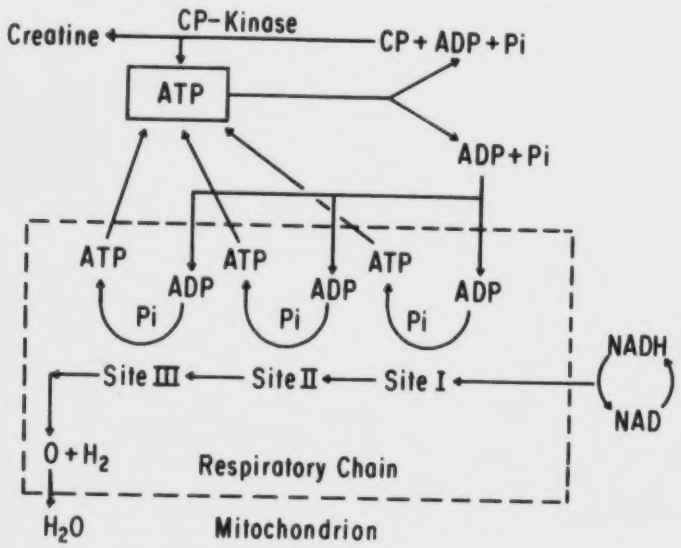


Figure 1. Interaction of CP, ADP, and CP-kinase.

The CP - CP-kinase phosphorylation of ADP to ATP is rapid and will sustain ATP at normal levels so long as mitochondrial function continues to produce ATP at a near-normal level. In low-level hypoxia, a 40% to 50% drop in CP will be observed prior to significant decrease in brain ATP concentration. If, however, the respiratory chain is inhibited (e.g., from a lack of oxygen acceptor at the terminal end, or a lack of phosphate acceptor, ADP, at sites I, II, and III in the chain) then the contribution of mitochondrial ATP production to the intracellular ATP concentration would be lost. ATP concentration would decrease, but not as fast as CP, since the CP - CP-kinase reaction rate could not keep up with the ATP utilization rate. In a seperate

experiment, inhibition of flow down the electron transport chain was established by decapitating the head of an anesthetized rat and waiting 30 seconds prior to immersion in liquid nitrogen. These data along with control data are shown in Table 1. In this experiment, the oxygen present in the brain and vascular system at the time of decapitation would have to be utilized prior to any significant drop in ATP. However, 30 seconds post-decapitation, ATP had decreased by 32.4% when CP decreased by 75.8%.

TABLE 1. EFFECT OF 30 SECOND AUTOLYSIS ON ATP AND CP IN THE RAT BRAIN

	ATP (%)	CP (%)
Control (Sham exposure)	100.0 ± 1.0 (45)	100.0 ± 2.4 (45)
30-sec autolysis	67.4 ± 2.4 (6) P < 0.001	24.2 ± 2.4 (6) P < 0.001

Mean ± SEM.
(n) = number of animals.
Control values: 2.50 ± 0.025 μmoles ATP/g; 3.38 ± 0.08 μmoles CP/g.
P = probability of being same as control population by Student's t-test for two group comparisons.

The results of the earlier work [12] where brain of rat was exposed in situ to 591 MHz microwaves (CW, 13.8 mW/cm^2 surface power density) are shown in Figure 2 where NADH fluorescence, [CP] and [ATP] are shown for sham exposed and microwave exposed brains. It was obvious from this work that microwave exposures increased NADH fluorescence and decreased [CP] and [ATP] in rat brain.

The work in this report was performed to determine the changes in rat brain NADH fluorescence, ATP and CP levels due to the effects of:

1. temperature alone: testing 35-42°C
2. differing exposure power levels: testing 0.00 (sham), 0.1 to 60 mW/cm^2 at three frequencies: 200, 591 and 2450 MHz, CW
3. amplitude modulation: testing changes in frequency between 4 and 32 Hz of the sinusoidal waveform during a microwave exposure of 13.8 mW/cm^2 at 591 MHz
4. pulsed modulation: testing 5 μsec/pulse at 250 and 500 pulses per second, also at 591 MHz

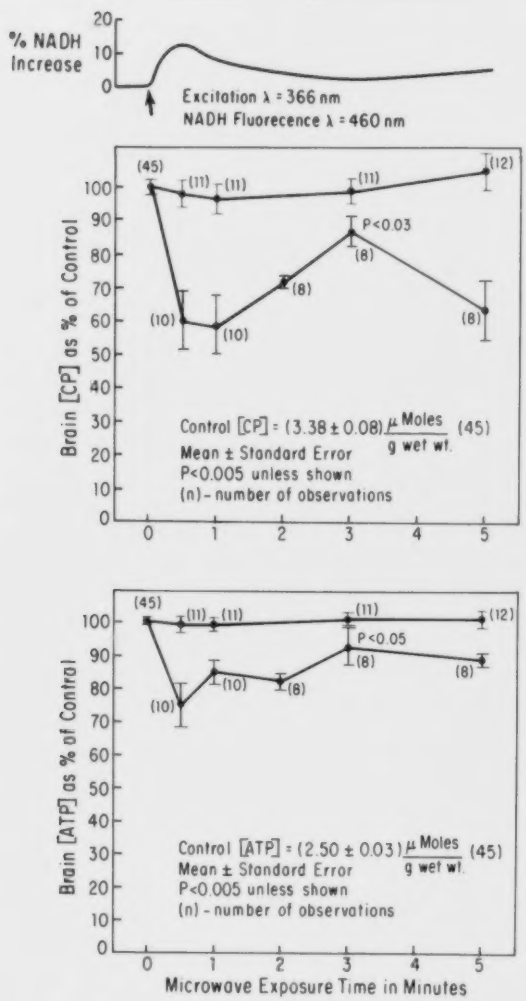


Figure 2. Levels of brain NADH, CP and ATP (relative to control) of sham and microwave-exposed (13.8 mW/cm^2 at 591 MHz, CW) rat head in situ [12]. Arrow at time zero indicates time at which microwave exposures were initiated. P values are the probability of being from the same population as controls as determined by the Student's t-test (two tailed) for two group comparisons. Reprints from Bioelectromagnetics 1, 1980 by A.P. Sanders, D.J. Schaefer and W.T. Joines with permission of Alan R. Liss, Inc.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

1. Microwaves at 200 and 591 MHz, 13.8 mW/cm^2, CW exposures have a direct effect on brain energy metabolism, as indicated by increased [NADH]and decreased [ATP], that is independent of tissue hyperthermia.

2. Studies of brain energy metabolism by relative NADH fluorescence, and [ATP] and [CP] assays at 200, 591 and 2450 MHz exposures indicated frequency specific effects.

3. Sine wave amplitude modulation (16 Hz), pulsed and CW microwave exposures produced the same inhibition in brain energy metabolism.

4. Using sine wave amplitude modulation, there was a greater effect on brain NADH fluorescence at 16-24 Hz than at 4, 8, 12, 28 and 32 Hz. This suggests a small modulation frequency effect.

5. The data support the conclusion that the observed microwave effects on rat brain NADH fluorescence, [ATP] and [CP] were primarily a function of carrier frequency and internal electric field intensity.

6. The data support the hypothesis that microwaves at specific frequencies inhibit electron transport chain function of brain mitochondria by inducing molecular dipole oscillation of active molecules whose biochemical function(s) depend(s) on stereospecificity. Such interactions could: 1) occur at multiple sites along the chain, 2) be frequency specific at a different frequency for each site, and 3) could occur at enzyme interaction sites throughout the cell. The threshold for the change in NADH fluorescence observed at 200 and 591 MHz, CW exposures as a function of electrical field strength indicates that these changes may be primarily a function of E field interaction with a single site of metal-active enzyme or transferase inhibition. The frequency at which microwaves induce dipole oscillations would be a function of the relaxation frequency(ies) of the involved molecule(s) which function in required stereospecific activity(ies) in biochemical reaction(s). Such microwave interactions would be expected to inhibit the reaction rate of the biochemical function.

Recommendations:

1. The effect of low power microwave radiation (0.1 - 10 mW/cm^2) on brain energy metabolism particularly need to be examined, since these are within the non-thermogenic range and now considered safe for human exposure.

2. Extensive work should be pursued to test the hypotheses presented in the previous section over the frequency range where such effects might be anticipated, i.e., 1 - 800 MHz.

SECTION 3

EXPERIMENTAL PROCEDURES

Animal Preparation and Instrumentation

Male Sprague Dawley rats, 175-225 g, were anesthetized with urethane (1250-1350 mg/kg, IP injection). The scalp and muscle at the top and side of the skull were removed from the brain area to be studied. A 5 x 5 mm aperture was made through the skull, leaving the dura intact. Animals exposed to 591 MHz microwaves were positioned under a titania-loaded horn antenna (3 x 3 cm) so that only the head and neck were exposed, as previously described [12]. Animals exposed to 200 and 2450 MHz microwaves were positioned in a microstrip irradiation system, as follows: the head was held rigid in a teflon head holder and located directly below a 1.5 cm diameter hole in the upper plate of the microstrip system. This system permitted continuous NADH fluorescence measurements before, during and after microwave exposures. A group of animals were studied in the microstrip system at 591 MHz to confirm the reproducibility of results obtained with the horn antenna exposures in the microstrip system.

The optical head of the 366 nm excitation light source was focused to a 1-2 mm spot on the cerebral cortex of the rat brain. A fiber optic bundle with plastic tips was positioned approximately 45° to the excitation light beam and at a distance of 1 to 2 cm from the focal spot on the cerebral cortex. The fiber optics were divided approximately 15 cm from the end, and each end attached to a filter-photomultiplier-amplifier assembly. One of these was a 460 ± 5 nm half band width (HBW) filter system for measuring NADH fluorescence, the other a 549 ± 2 nm HBW interference filter system for recording general brain fluorescence, allowing correction of measurements for the effect of increased hemoglobin in the field on NADH fluorescence, as detailed by Kramer and Pearlstein [13].

An AIL 125A microwave generator was used to produce the 200, 591 and 2450 MHz exposures. This was connected to a circulator (Western Microwaves Models 3J-1997, 3JA-2086, or 3JA-2040), a dual direction coupler (NARDA-Model 3020A or 3022) (with sensors to the two HP 435A power meters for measuring power transmitted to the antenna or microstrip system, and the reflected power), an antenna, or microstrip system, and a Termaline Model 8135 Coaxial resistor.

Calculation and Measurement of Microwave Power Absorbed by Test Animal

For applying microwave energy at frequencies between 200 MHz and 2500 MHz, we have used an air-filled stripline of our own construction and a waveguide horn antenna previously described [12]. The stripline (also known as microstrip) consists of two parallel planes, the width and spacing of

which yield a 50-ohm characteristic impedance at any plane perpendicular to the direction of energy propagation. Most of the energy will propagate in a transverse electromagnetic (TEM) wave between the stripline planes as long as the spacing between planes is less than one-half of the wavelength. Thus a line with 6 cm between planes was used at all frequencies up to 2500 MHz. The problem of generation of higher-order modes when a test animal is placed in the line, thus potentially lowering the upper frequency limit of the stripline, will be discussed later in this report.

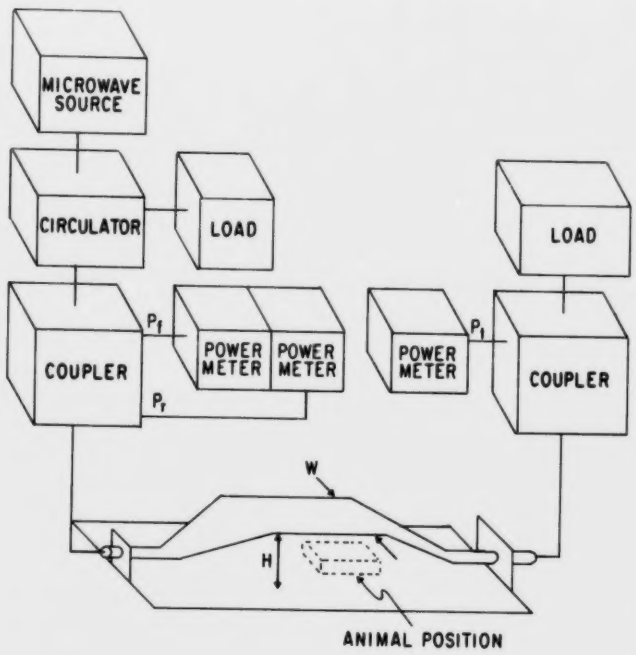


Figure 3. Microstrip transmission line irradiation system. The conductor of width (W = 29.5 cm) is a sheet of 0.16 cm aluminum spaced a height (H = 6.0 cm) above an aluminum ground plane in the region where the animal will be placed. W and H are adjusted in other regions to maintain a 50-ohm characteristic impedance throughout.

A diagram of the microstrip system is shown in Figure 3. During a typical exposure with this system, the test animal is placed within the rectangular region indicated. Before the animal is placed in the line, a uniform TEM field (similar to the far-field of an antenna) exists between the conductors spaced 6 cm apart. The width of the uniform field region where the animal will be placed is about 1.3 x height (H), or 7.8 cm for the line in Figure 3. Fiber optic light guides for the time-sharing fluorimeter are located immediately above a small hole (1.5 cm diam.) cut in the con-

ductor plate (width = 29.5 cm). The hole causes a small but negligible perturbation of the field.

Whole-Body Specific Absorption Rate

The forward (P_f), reflected (P_r), and transmitted (P_t) powers can be measured by the power meters as indicated, so the total absorbed power in the test animal (P_a) plus the stripline absorption (P_{as}) is

$$P_a + P_{as} = P_f - P_r - P_t \text{ watts} \quad (1)$$

With the test animal removed and P_f kept the same, P_{as} should remain about the same, as we have

$$P_{as} = P_f - P'_r - P'_t \text{ watts} \quad (2)$$

Subtracting (2) from (1) yields P_a as

$$P_a = P'_r - P_r + P'_t - P_t \text{ watts} \quad (3)$$

From this measured value of P_a, the whole-animal SAR in mW/g is determined by dividing P_a by the weight of the animal.

The amount of forward power (P_f) required to produce a given SAR in the test animal within the transmission line can be estimated in this way. If an SAR of 2.5 mW/g is desired for a 320 g rat, then the animal must absorb 0.8 W. Approximately 60% of the forward power within the 200-2500 MHz range will be reflected from the surface of the animal. Hence, the forward power incident upon the animal alone will need to be about 2 W to yield an absorption of 0.8 W. The absorption cross-section of the animal in the line is greater than his actual cross-section [14] and may be around 16.5 cm^2 for a 320 g rat facing the microwave source. Hence, the power density required is 121 mW/cm^2, and the total forward power (P_f) is estimated to be 32.8 W to produce an SAR of 2.5 mW/g in a 320 g rat. Such estimates were used to guide prediction of ranges of P_f required, but precise calculations were completed for actual experiments.

A problem with using equations (1), (2) and (3) to determine the total power absorbed in the animal is that they do not take into account the power scattered out of the microstrip line due to the presence of the animal. Hence, the method is accurate only for closed systems in which the power is transmitted to the load, reflected back to the source, absorbed in the line, or absorbed in the animal. Since the total power scattered out of the microstrip line would be impossible to measure, we did not use this method to determine P_a.

Rat-brain Dosimetry in Microstrip Line

We used the following method to calculate and measure power absorbed at a given point within the brain of the test animal. For a long biological sample in the microstrip line, as for a 320 g rat, the power transmitted longitudinally through the animal is zero. Hence, from equation (11) of Joines et al. [14], we find at depth z within the test animal from surface nearest the source:

$$\frac{P_a(z)}{P_i} = \frac{2}{\delta}\,(1 - |\rho|^2)\,(1 + \Delta w_s/w_s)\,e^{-2z/\delta}\,\frac{mW/g}{mW/cm^2} \tag{4}$$

where δ is the depth of penetration given by [15]

$$\delta = \frac{2\mathrm{Re}\eta}{\sigma|\eta|^2} \tag{5}$$

where ρ is the reflection coefficient given by

$$\rho = \frac{\eta-\eta_0}{\eta+\eta_0} \tag{6}$$

where η is

$$\eta = \frac{\eta_0}{\sqrt{\varepsilon^*_r}} \tag{7}$$

η_0 is 377 ohms, ε^* is the relative complex permittivity of the biological tissue, and σ is the conductivity of the tissue.

For a 320 g rat facing the source and parallel to the propagation direction, the height and width of the animal are approximately h = 2.13 cm and w_s = 4 cm. With H = 6 cm, we find Δw_s = 1.1 cm from equation (4) of [14], or from Figure 4, which is plotted from equation (4) of the reference cited. Thus (4) becomes

$$\frac{P_a(z)}{P_i} = \frac{2.55\,(1 - |\rho|^2)\,e^{-2z/\delta}}{\delta} \tag{8}$$

For brain tissue, δ and $|\rho|^2$ vary with frequency [16]. For example, Figure 5 shows δ = 7.7 cm, $|\rho|^2$ = 0.69 at 200 MHz; δ = 6 cm, $|\rho|^2$ = 0.61 at 400 MHz; and δ = 5.1 cm, $|\rho|^2$ = 0.55 at 600 MHz. Using these values and equation (8) we calculated power absorbed at a depth z = 1 cm for three frequencies:

$$\frac{P_a(z)}{P_i} = 0.079\,\frac{mW/g}{mW/cm^2} \text{ at } 200 \text{ MHz},$$

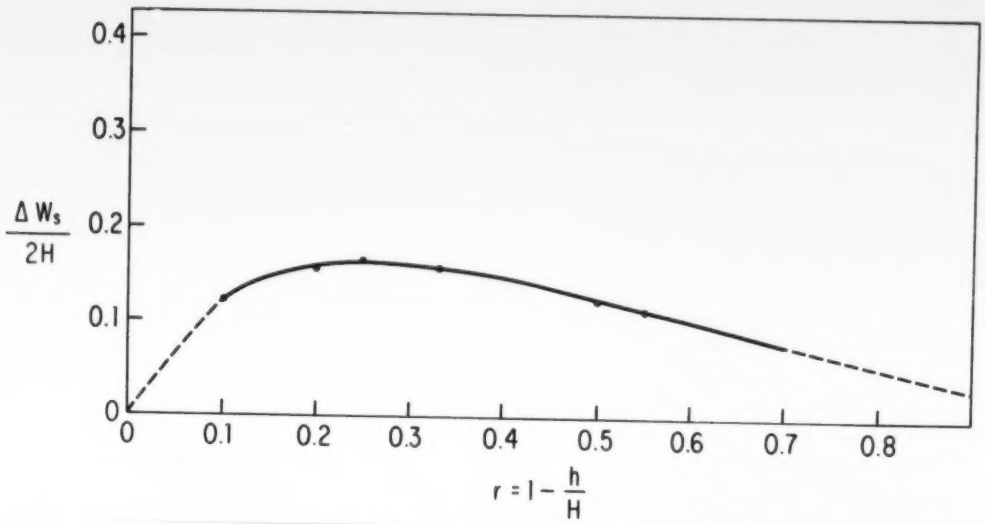


Figure 4. Apparent width increase of a rectangular sample of height h and width w_s in a uniform field of height H.

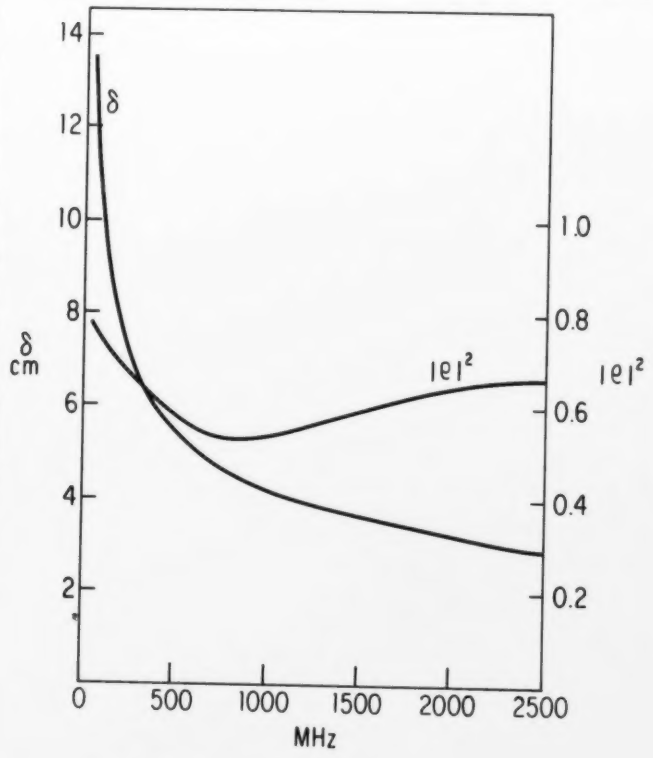


Figure 5. Variation of depth of penetration (δ) and reflection coefficient (ρ) with frequency.

$$\frac{P_a}{P_i} = 0.120 \frac{mW/g}{mW/cm^2} \text{ at 400 MHz,}$$

$$\frac{P_a}{P_i} = 0.152 \frac{mW/g}{mW/cm^2} \text{ at 600 MHz.}$$

Figure 6 shows the values measured at about the same depth within a dead rat using a Vitek model 101 probe compared with our calculated values using equation (8). The density of absorbed power at a site within the rat brain was estimated from the relationship:

$$P_a = Kdc\Delta T/t \qquad (9)$$

where P_a is the density of absorbed power in W/cc; K is 4.186 joules/cal, d is the tissue density in g/cc; c is the specific heat of tissue in cals/g/°C; ΔT is the temperature change in °C; and t is the time in seconds.

From equation (9), if the temperature increase versus time is plotted from a given P_a, the initial slope of the curve is P_a divided by Kdc. By recording the temperature increase versus time at a 1 cm depth within the brain of the animal during a fixed level of irradiation, and using the values of 1.05g/cc and 0.88cal/g/°C for the density and specific heat found in brain tissue [17], we derived densities of absorbed power and SAR from the $\Delta T/t$ values recorded.

Each point in Figure 6 represents the average of data measured on three animals. At each frequency the incident power density (P_i) was kept at or near 100 mW/cm^2. The animals were typically heated for about 30 minutes and ΔT was about 10°C.

Discussion and Results and Prediction of Waveguide Modes

The rectangular sample has been assumed to be infinitely long in equation (4). This assumption is valid if the front and back surfaces of the sample are isolated by 20 dB or more of attenuation, which occurs when $L/\delta \geq 2.31$ (as derived in reference [14]), where L is the sample length and δ is penetration depth. For a 320 g rat, L is about 20 cm, and L/δ exceeds 2.31 at all frequencies above about 100 MHz.

Equation (4), which is plotted as the solid curve in Figure 6, does not take into account resonance effects due to animal size and shape. However, the measured SAR, or P_a/P_i, exhibits a resonance peak at 600 MHz. In fact, up to about 800 MHz, the measured SAR follows the pattern of SAR versus frequency obtained for the free-field exposure of a prolate spheroid model of a medium-sized rat (320g). The difference is that our measured results are shifted downward in frequency so that the resonance peak occurs at 600 MHz instead of about 750 MHz, as given by the RF Dosimetry Handbook [18]. Since in our situation the animal is on a ground plane, the downward shift of

frequency is expected. The downward shift would be by a factor of two if the animal were, in fact, a perfect prolate spheroid and in perfect contact with the ground plane. Of course, the animal's actual shape, and the insulating effect of the fur prevent the resonant frequency from being half the free-field value.

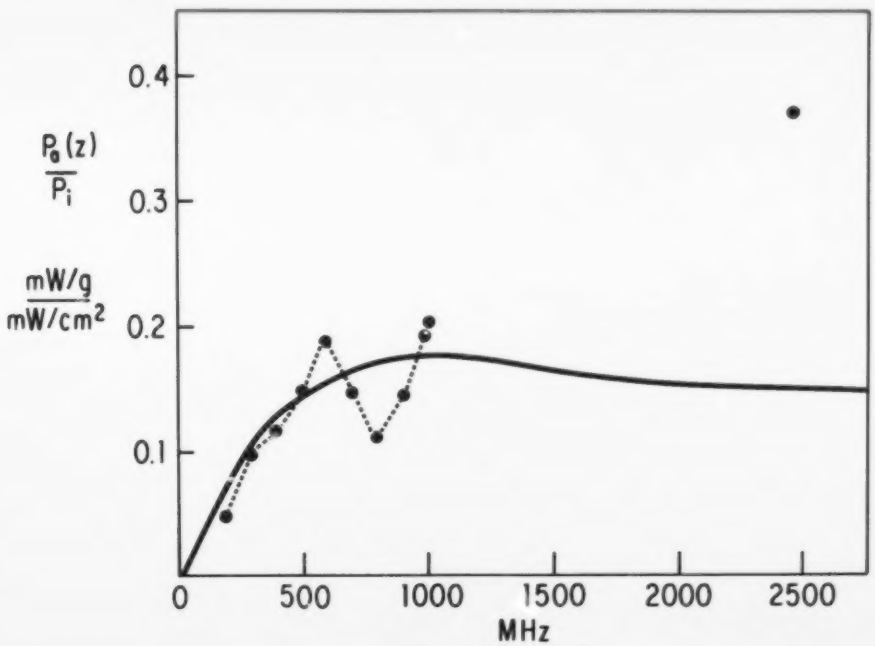


Figure 6. $\frac{P_a(z)}{P_i}$ vs frequency at z = 1 cm in rat brain.
calculated———— measured----------

Above 800 MHz the measured SAR in Figure 6 is not predictable by our calculations or by the RF Dosimetry Handbook. At frequencies above 800 MHz we conclude that higher-order modes (higher than TEM) or waveguide modes exist within the line when the test animal is present. The lowest order waveguide mode that can exist in the microstrip when the height H in Figure 1 is electrically equal to one-half wave length. With no animal in the line, frequency, velocity and wave length are related by

$$f = \frac{v_0}{\lambda} = \frac{3 \times 10^{10}}{\lambda} \text{ cm/sec} \qquad (10)$$

and the frequency at which H = λ/2 is

$$f = \frac{3 \times 10^{10} \text{ cm/sec}}{2 \times 6 \text{ cm}} = 2500 \text{ MHz}$$

With the animal of height h and relative dielectric constant ε_r in the line, the electrical height changes from H to H' given by

$$H' = H - h + h\sqrt{\varepsilon_r} \tag{11}$$

Hence, the frequency at which H' = λ/2 is

$$f' = \frac{v_o}{2H'} = \frac{v_o}{2(H-h) + 2h\sqrt{\varepsilon_r}} \tag{12}$$

Taking ε_r = 49 for brain tissue, and h = 2.13 cm (as used earlier) we have

$$f' = \frac{3 \times 10^{10} \text{ cm/sec}}{2(3.87) + (4.26)(7)} = 800 \text{ MHz} \tag{13}$$

At this frequency and above, therefore, waveguide modes of propagation would be expected to exist within the line, thus complicating SAR determinations.

It is worth emphasizing that waveguide modes between 800 MHz and 2500 MHz can only exist along the approximately 25-cm section of line occupied by the body of the 320 g rat. The fact that the entire body of the rat is within the line and oriented along the direction of propagation serves to introduce waveguide modes at the relatively low frequency of 300 MHz. If only the head of the rat had been protruded into the line, much of the complication in determining SAR above 800 MHz would have been avoided.

Rat Brain Dosimetry Calculation and Measurements for Horn Antenna

HP-435A power meters were used to measure microwaves at 591 MHz applied through a dual-directional coupler (HP-778D) to a dielectric-loaded (titania) horn antenna. The design and construction of this antenna has been described previously [12]. This 3 cm by 3 cm aperture antenna was positioned about 3 cm above the rat brain surface, so that the microwaves were incident normal to the top surface of the brain, and the electric field was along the body axis as shown in Figure 7. With the 3 cm spacing, the electric and magnetic field intensities were approximately constant on a plane perpendicular to the direction of energy propagation; that is, the field is approximately a uniform plane wave at the tissue surface. From measurements made using a RAHAM Model 2 hazard meter, the radiation pattern from the horn antenna was such that the head of the animal was uniformly irradiated, and the torso was subjected to incident power densities less than 0.1 mW/cm^2. This left the nose and neck of the animal nonuniformly irradiated.

A radiation detection meter equipped with diode probe (General Microwave Model RAHAM 2) was used to measure the surface power density at the

position of the tissue surface in front of the horn before the animal was placed in the field. With the measured value of P_i, the absorbed power density (P_a in mW/g) for the size and shape of exposed tissue may be determined from published equations and curves [18].

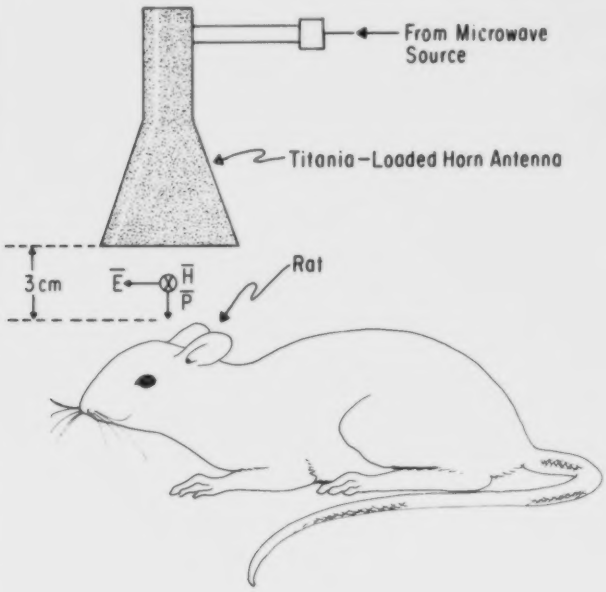


Figure 7. Horn antenna exposure arrangement. $\bar{E}$ is the vector electric field intensity, $\bar{H}$ is the vector magnetic field intensity, and $\bar{P}$ is the Poynting vector of instantaneous power density.

A prolate spheroid with electric field along the major axis is a reasonable simulation of the volume of rat brain under our conditions, since the nose and neck of the animal are partly within the irradiation field of the antenna. This means that the total irradiated volume is not precisely known. To establish guidelines on SAR, we used curves from the Radiofrequency Radiation Dosimitery Handbook [18]. An irradiated volume of 110 cm^3, in the form of a prolate spheroid with semiaxis a = 7 cm and b = 1.94 cm, at 591 MHz produces a SAR of 0.42 $mW/g/mW/cm^2$ when the electric field is along the a-axis. This value of SAR and volume probably represents an upper limit. As a lower limit, an irradiated volume of 25 cm^3, with a = 3.8 cm and b = 1.25 cm, at 591 MHz produces an SAR of 0.18 $mW/g/mW/cm^2$ when the electric field is along the a-axis. Thus, values of whole-body SAR are comparable but less than the local SAR values determined from temperature versus time measurements as discussed below.

The density of absorbed power at a site within the rat brain may be estimated from equation (9) by recording the temperature increase versus

time near the mid-brain of the animal during a fixed level of irradiation. This method was used to determine P_a. A hypodermic-type thermistor probe (YSI, 22 guage) was used to record the temperature. The probe was oriented at right angles to the electric-field vector in air to minimize artifacts in the measurement of brain temperature. These measurements of $\Delta T/t$ were made using a thermistor probe at a time before the VITEK probe was available to us However, the measurements were made at the same site in the rat brain as for the stripline exposures.

Using the values of 1.05 g/cc and 0.88 cal/g/°C for the density and specific heat found in brain tissue [17], we calculate densities of absorbed power and SAR from the $\Delta T/t$ values recorded on a dead rat at two levels of P_i. Specifically, $\Delta T/t = 0.010$°C/sec at $P_i = 60$ mW/cm^2, and $\Delta T/t = 0.017$°C/sec at $P_i = 100$ mW/cm^2. Thus for 0.010°C/sec:

$$P_a = (4.186)(1.05)(.88)(0.010)$$

$$= 38.7 \text{ mW/cc or } 36.8 \text{ mW/g}$$

and

$$\text{SAR} = \frac{36.8 \text{ mW/g}}{60 \text{ mW/cm}^2} = 0.61 \frac{\text{mW/g}}{\text{mW/cm}^2}$$

Likewise, for 0.017°C/sec:

$$P_a = 65.7 \text{ mW/cc or } 62.6 \text{ mW/g}$$

and

$$\text{SAR} = \frac{62.6 \text{ mW/g}}{100 \text{ mW/cm}^2} = 0.626 \frac{\text{mW/g}}{\text{mW/cm}^2}$$

The measured specific absorption rates (mW/g/mW/cm^2) for the three designated frequencies are summarized in Table 2.

TABLE 2. MEASURED LOCAL SAR AT 1 CM DEPTH IN RAT BRAIN DUE TO IRRADIATION BY MICROSTRIP AND HORN ANTENNA

Frequency (MHz)	SAR (mW/g/mW/cm^2)	
	Microstrip	Horn Antenna
200	0.046	
591	0.185	0.62
2450	0.368	

The microstrip data in Table 2 are taken from the measured points in Figure 6. At 591 MHz, the local SAR due to the horn antenna is 0.62/0.185 = 3.35 times greater than for the microstrip. This difference is due to the different orientation of the animal in the irradiation field of the two systems.

SECTION 4

STUDY 1. THE EFFECT OF INCREASED TEMPERATURE ALONE AND WITH MICROWAVE EXPOSURE ON BRAIN ATP, CP AND NADH FLUORESCENCE LEVELS

Rats were prepared as described above and placed between two heating pads which received their power from a Yellow Spring Instrument Model 514 temperature measuring and regulating unit. A YSI 22 guage thermistor was placed at a depth of 2 to 3 mm beneath the surface of the brain. The unit was adjusted to maintain brain temperature within 0.1°C of the desired temperature, i.e., 35.6, 37, 39, 40 or 41°C.

The measurement of brain NADH fluorescence is complicated by the extreme variability of the location of blood vessels immediately above, and on the surface of the cerebral cortex. Since hemoglobin absorbs both excitation light (366 $\pm$ 5 nm) and fluorescence light (460 $\pm$ 5 nm and 549 $\pm$ 2 nm), the wide variation between rats in blood vessel location on the brain surface causes a wide variation among animals in the NADH fluorescence level and microwave induced magnitude of change in NADH fluorescence. As a result of this, each animal must serve as its own control. Similarly it is not valid to determine mean values and standard deviation as a group due to this wide variation in blood vessels among animals and the location of the excitation-fluorescence light focal spot. Consequently all figures showing NADH fluorescence levels as a function of temperature or microwave exposure reflect the data obtained from a single animal. No less than 6 animals were used for each condition studied to ascertain the microwave effects on NADH fluorescence level of the brain.

The effects of increasing rat brain temperature on NADH fluorescence with the temperature feedback heater-regulator system detailed above are shown in Figure 8 for one animal anesthetized with sodium pentobarbital (I) and four animals anesthetized with urethane. Barbituate anesthesia has been shown to affect brain energy metabolism [20]. Thus it was not surprising that in pentobarbital anesthetized animals a decrease in NADH fluorescence occurred as temperature increased. The urethane anesthetized animals showed no significant, consistent change in NADH fluorescence as the brain temperature was increased from 35 to 42.5°C, as indicated in II through V on Figure 8. There is no obvious explanation for this difference in effect of the two anesthetic agents. A brain temperature of 35.6°C was selected as the control brain temperature, and urethane anesthesia was used for all experiments in this report. Ten urethane anesthetized control animals with brain temperature of 35.6°C were analyzed for brain ATP and CP content. The resultant CP and ATP values for the 10 rats were 3.44 $\pm$ 0.11 and 2.47 $\pm$ 0.03 μmoles/g respectively.

When comparing this data with that from Table 1, obtained using pentobarbital, no difference in brain ATP and CP levels could be detected between the urethane or sodium pentobarbital anesthetized normal brain at 35.6°C temperature.

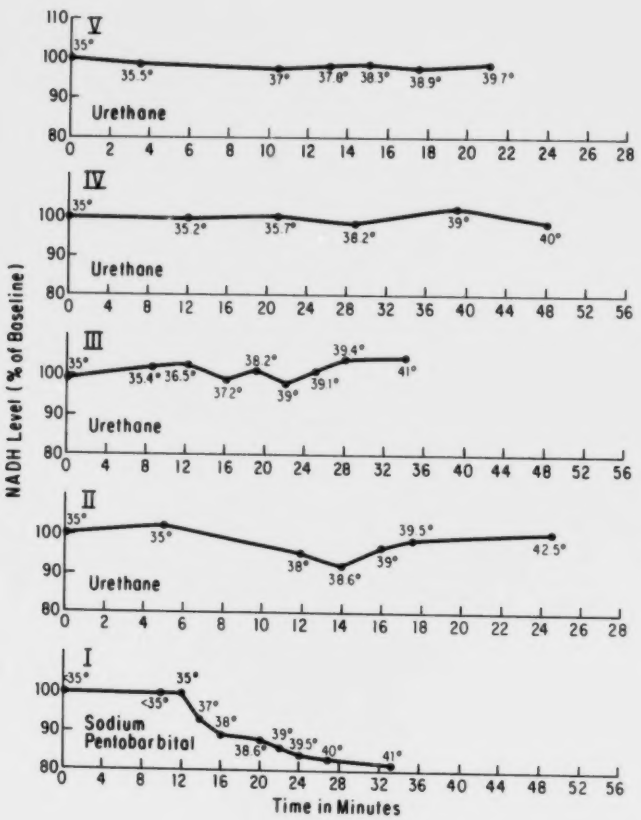


Figure 8. NADH fluorescence vs change in brain temperature only.

Variations in levels of ATP and CP as a function of temperature are shown in Table 3 where the 35.6°C values for ATP and CP concentrations were considered control values. We are unable to explain why the NADH fluorescence did not decrease in the urethane anesthetized animals when [ATP] and [CP] decreased.

TABLE 3. RAT BRAIN [ATP] AND [CP] AS A FUNCTION OF TEMPERATURE

°C Temperature	ATP (μmoles/g)	% of control	CP (μmoles/g)	% of control	n
35.6	2.50 ± 0.03	100 ± 1.2	3.41 ± 0.09	100 ± 2.6	51
37.0	2.35 ± 0.03	94 ± 4.0 N.S.	2.74 ± 0.15	80.4 ± 4.4 P< 0.001	10
39.0	2.23 ± 0.09	89.2 ± 3.2 P< 0.01	2.43 ± 0.08	71.3 ± 2.3 P< 0.001	15
41.0	1.77 ± 0.03	70.8 ± 1.2 P< 0.001	1.50 ± 0.11	44.0 ± 3.2 P< 0.001	10

Values reported are Mean ± SEM
Abbreviations: n = number of animals; P = probability that there is no difference between tested and control groups by unpaired Student's t-test; N.S. = not significant

Based on these data we selected a temperature of 39.0 ± 0.1°C as representative of an isolated temperature effect and compared [ATP] and [CP] in these animals with the [ATP] and [CP] levels in: (1) normothermic rat brains exposed to microwave and (2) hyperthermic (39°C) rat brains exposed to microwaves. The effects of hypoxia on normothermic rat brain were also examined.

Effects of Microwaves and Temperature on Rat Brain Energy

Rat brain with temperature regulated to 39.0 ± 0.1°C was exposed to microwaves (591 MHz, CW, 13.8 mW/cm^2) for 0.5, 1, 3 and 5 min. Another group of animals with brain temperature at 35.6°C were subjected to 2% O_2 + 98% N_2 exposures for 0.5, 1 and 2 min. The rat brains from these groups were assayed for ATP and CP concentration (See Appendix I for methodology). The results of these experiments and NADH fluorescence curves are shown in Figure 9 where the hyperthermia and hypoxia-tested animals are compared with those where temperature was held at 35.6°C [12].

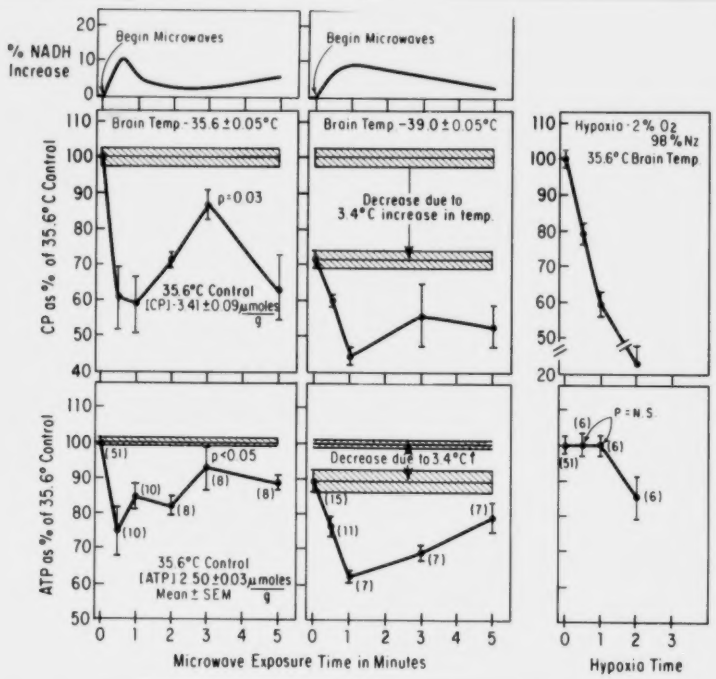


Figure 9. Rat brain [ATP] and [CP] normoxic at 35.6 and 39°C with and without microwave exposure, and hypoxic (2% oxygen) at 35.6°C. Unless shown $P < 0.01$. Microwave exposures are at 591 MHz.

The NADH fluorescence curve for the 39°C brain immediately showed an increase for all exposures, reached a maximum at 0.9 min and slowly decreased through 5 min. The marked increase in NADH fluorescence when the microwaves were turned on was common to both exposed brain temperatures. These data show that 0.5 and 1 min exposures to the 35.6°C brain resulted in decreases in [ATP] and [CP] that were significantly greater than the decrease in [ATP] and [CP] of the brain caused by increasing temperature by 3.4°C. Since the conditions of microwave exposure (591 MHz, CW, 13.8 mW/cm^2) did not produce generalized tissue hyperthermia, we conclude that there was a direct effect of microwaves on the inhibition of cellular metabolism [12].

Effects of Hypoxia on Normthermic Rat Brain

In the hypoxia experiment the brain [ATP] remained constant even though there were 21 and 41% decreases in [CP] in the brain after 0.5 and 1 min, respectively. The CP - CP-kinase function of transferring the high energy phosphate bond from CP to ADP to yield ATP and the mitochondrial production of ATP in the 2% O_2 together were able to

sustain the brain [ATP] within normal limits until [CP] fell below 60% of control levels. However, with a 0.5 min exposure to microwaves [ATP] fell to 74.8% of control even though [CP] values remained at a level (60.4%) expected to maintain normal level of ATP. This observation supports the concept that there was direct microwave inhibition of mitochondrial function. The 35.6°C brain [ATP] and [CP] which are shown as a function of time of microwave exposure were data obtained using sodium pentobarbital anesthesia. Prior to deciding this, groups of 6 urethane anesthetized animals were subjected to the same microwave exposure (591 MHz, CW, 13.8 mW/cm^2 surface power density) for 0.5, 1, 3 and 5 min. Immediately after each exposure, the brain was frozen. removed and assayed for ATP and CP concentration. None of the ATP and CP levels at each microwave exposure interval deviated by more than 4.5% from the data for the corresponding microwave exposure interval in the sodium pentobarbital anesthetized animals. Consequently the graphs already obtained from the sham or microwave exposed, 35.6°C brain, sodium pentobarbital anesthetized animals were used in Figure 9.

The 39° unexposed brain showed a significant decrease in [ATP] even though the [CP] was decreased only 71.3% of the 35.6°C control brain level. This was probably due to an increase in the metabolic rate of the brain such that the increased utilization of ATP was not matched by adequate increases in the mitochondrial rate of production or CP-kinase function. Thus new steady state levels of CP, ATP and ADP would be reached with the temperature-increased metabolic activity of the cell.

When rat brains at 39°C were exposed to microwaves with the horn antenna at 591 MHz, CW, 13.8 mW/cm^2 surface power density for 0.5, 1, 3 and 5 min, significant decreases in brain [ATP] and [CP] were seen. The microwaves resulted in decreases in [ATP] when the decreases in [CP] (74 - 84% of control) were at levels where the CP + ADP + CP-kinase→C + ATP-system would be expected to sustain normal [ATP] (Table 4).

TABLE 4. EFFECT OF MICROWAVE (591 MHz,CW) ON 35.6 AND 39°C RAT BRAIN

Exposures	35.6°C Brain CP (µmoles/g)	35.6°C Brain ATP (µmoles/g)	n	39°C Brain CP (µmoles/g)	39°C Brain ATP (µmoles/g)	n
Control	3.41 ± 0.09	2.50 ± 0.03	51	2.43 ± 0.09	2.23 ± 0.08	15
	% of control	% of control		% of control	% of control	
	100 ± 2.6	100 ± 1.67	10	100 ± 3.7	100 ± 3.6	11
0.5 min	60.6 ± 8.7	74.8 ± 6.6	10	84.4 ± 2.4	86.0 ± 4.6 P < 0.03	7
1 min	58.9 ± 8.6	84.9 ± 3.4	10	74.9 ± 2.9	70.2 ± 1.4	7
3 min	86.9 ± 4.2 P < 0.03	92.6 ± 6.2 P < 0.05	8	78.6 ± 2.3	77.5 ± 2.2	7
5 min	63.6 ± 8.9	88.8 ± 1.9	8	74.1 ± 8.2	88.7 ± 4.5	7

Mean ± SEM; P < 0.01 unless shown for all exposures by unpaired Student's t-test.

STUDY 2. THE EFFECTS OF DIFFERENT MICROWAVE POWER LEVELS WITH CW EXPOSURE

The effects of different power levels with 200, 591 or 2450 MHz, CW exposures are shown in Table 5 and Figure 10. The 2450 MHz exposures caused no significant change in NADH fluorescence at SAR levels ranging from 0.18 to 14.72 W/kg. The 200 and 591 MHz exposures gave similar curves (Figure10) except that 5 of the 6 animals used at 200 MHz showed less than a 4.8% increase in NADH fluorescence. (Only 1 animal approached the magnitude of the change in fluorescence observed with the 591 MHz exposures.) The six 591 MHz exposed animals had brain NADH fluorescence changes, as a function of surface power density, randomly distributed between the minimum and maximum observed changes in NADH fluorescence. The minimum and maximum changes in NADH fluorescence are shown as a function of SAR and E-field strength in Table 6 for 200 and 591 MHz, CW exposures. For the same SAR, 200 MHz exposures at low SAR values consistently gave higher NADH fluorescence changes than the 591 MHz exposures. Similarly the 200 MHz exposed animals reached a maximum increase in NADH fluorescence at lower SAR values than the 591 MHz exposed animals. The SAR was 0.82 W/kg at 200 MHz to give maximum increase in brain NADH fluorescence. In contrast, the 591 MHz SAR value was 3.7 W/kg. Thus for equal SAR values below saturation levels the 200 MHz exposures consistently gave higher increases in brain NADH fluorescence than did 591 MHz exposures.

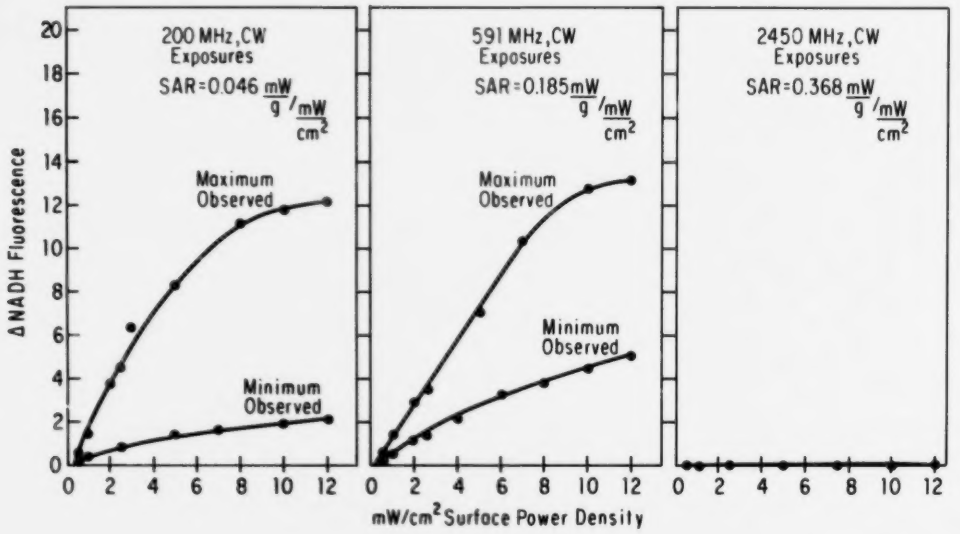


Figure 10. Change in NADH fluorescence of rat brain vs surface power densities at 200, 591, and 2450 MHz, CW exposures in microstrip system.

However, if the Δ% NADH fluorescence is plotted as a function of the E-field strength (V/m) one can estimate that the threshold for microwave induced fluorescence was essentially the same (3-4 V/m) for both frequencies (Figure 11). This would suggest that the tissue electrical field strength is the major parameter in the observed microwave effects of inducing increased NADH fluorescence levels [Allis, J: Personal communication]. This appears to be inversely related to decreased [ATP] and [CP] of the exposed rat brains. This also supports our hypothesis that microwaves inhibit electron transport chain function by inducing dipole oscillations(s) at the divalent metal-containing molecules, since the magnitude of microwave induced oscillations of the metal containing groups would be dependent upon the imposed electrical field strength. It is possible that only one metal containing active transfer site is being affected by both frequencies. However, the electron transport chain and associated enzyme systems have multiple divalent metal active sites. Each must function, without inhibition, for the electron transport chain to continue its function of oxidative phosporylation, i.e. ATP synthesis coupled to oxygen consumption. Each metal-active site could have a discrete natural resonance frequency and energy absorption requirement to have induced oscillation. This could be a function of molecular structural differences and rigidity of stereospecificity of its transfer function. Rigidly controlled experiments studying changes of tissue NADH fluorescence levels, [ATP] and [CP] will be required to determine

TABLE 5. THE EFFECT OF FREQUENCY, SAR AND E-FIELD ON MICROWAVE INDUCED CHANGES IN RAT BRAIN NADH FLUORESCENCE DURING A 2 MINUTE EXPOSURE

	200 MHz, CW				591 MHz, CW				2450 MHz, CW		
mW/(cm^2)	SAR W/kg	E V/m	Maximum Δ NADH*	Minimum Δ NADH	SAR W/kg	E V/m	Maximum Δ NADH	Minimum Δ NADH	SAR W/kg	E V/m	Maximum Δ NADH
0.5	0.02	6.25	+ 0.6	+0.15	0.09	11.3	+ 0.6	+0.25	0.18	10.4	0
1.0	0.04	12.5	+ 1.4	+0.35	0.18	22.5	+ 1.5	+0.55	0.36	20.7	0
2.0	0.09	25.0	+ 3.7	+0.66	0.37	45.0	+ 3.0	+1.20	0.73	41.4	0
2.5	0.11	31.3	+ 4.5	+0.80	0.46	56.3	+ 3.6	+1.55	0.92	51.8	0
5.0	0.23	62.5	+ 8.3	+1.40	0.92	113	+ 7.0	+2.80	1.84	104	0
10.0	0.46	125	+11.8	+2.00	1.85	225	+12.8	+4.55	3.68	207	0
20.0	0.82	250	+12.4	+2.50	3.70	450	+13.5	+4.80	7.36	414	0
40.0	1.80	500	+12.4	+2.50	7.40	900	+13.5	+4.90	14.72	828	0

*Δ NADH = percent change in NADH fluorescence.

TABLE 6. CHANGE IN RAT BRAIN NADH FLUORESCENCE VS SAR AT 200 AND 591 MHz, CW EXPOSURES

SAR W/kg	E-field V/m	200 MHz Maximum % increase	E-field V/m	591 MHz Maximum % increase	ΔNADH 200 / ΔNADH 591
0.25	90	8.7	35	2.2	3.95
0.5	137	12.0	62	4.0	3.00
1.0	175	12.4	115	7.5	1.65
1.5	200	12.4	185	11.2	1.11
2.0	250	12.4	230	12.8	0.97
		Minimum % increase		Minimum % increase	
0.05	14	0.4	5	0.1	4.00
1.0	25	0.71	13	0.27	2.63
0.2	50	1.20	25	0.62	1.93
0.3	75	1.56	40	0.96	1.62
0.4	100	1.80	47	1.31	1.37
0.5	237	2.06	60	1.60	1.29

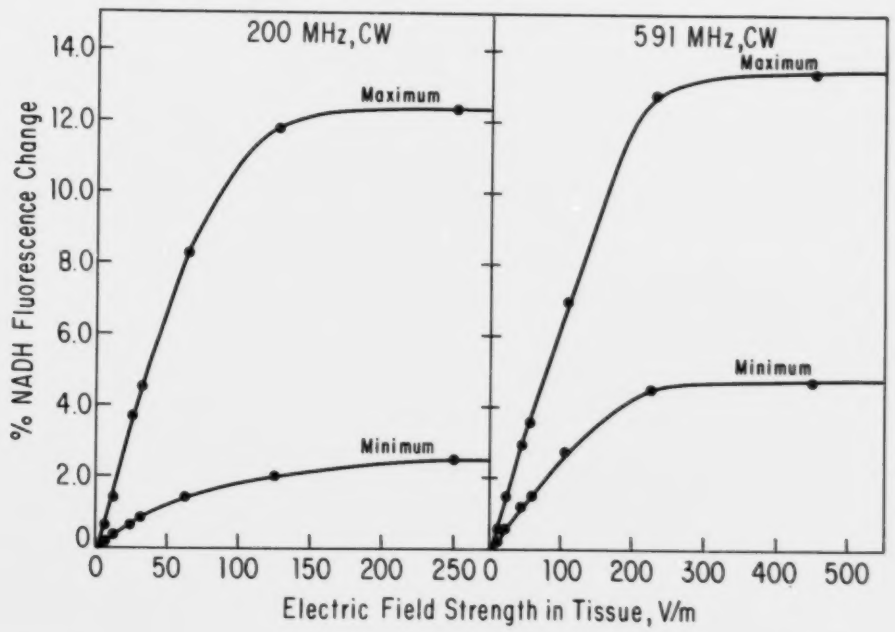


Figure 11. E field (V/m) vs Δ% NADH fluorescence of rat brain at 200 and 591 MHz, CW exposures.

if this is single site or multiple sites of microwave induced inhibition of brain energy metabolism.

The correlation of changes in rat brain NADH fluorescence, [ATP] and [CP] with duration of exposure are shown in Figure 12 for 200, 591 or 2450 MHz, 13.8 mW/cm^2 surface power density microwaves. An optimum curve of NADH fluorescence as a function of time of exposure is shown for each of the 3 frequencies. (Due to the wide variation in brain NADH fluorescence as a result of significant differences of brain vasculature observed through the skull aperture, it is essential that each animal serve as its own control. Thus change in NADH fluorescence can not be averaged at each exposure point, and a maximum and minimum curve were selected from the curves of animal studies at each frequency.) The changes in NADH fluorescence correlate with the changes in brain [ATP] and [CP] at the 200 and 591 MHz exposures.

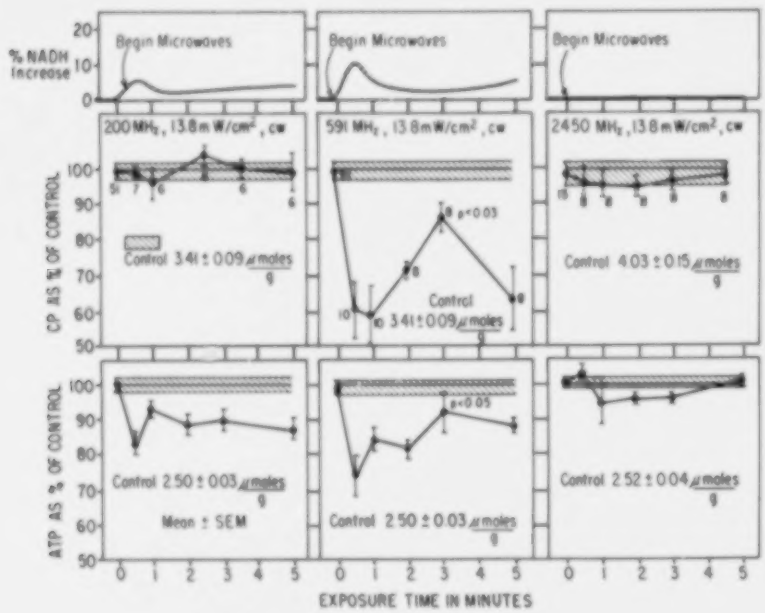


Figure 12. Rat brain NADH fluorescence, [CP] and [ATP] at 200, 591 and 2450 MHz, CW, 13.8 mW/cm^2 surface power density vs time of exposure.

The 2450 MHz exposures caused no significant changes in brain NADH fluorescence. It may be concluded from these data that the 2450 MHz frequency is too high to induce molecular oscillation(s) along the electron transport chain since no significant difference was observed in the different biochemical analyses. This is consistent with Pethig's [19] observation that microwave induced dipole oscillations in protein solution is observed mainly in the 30-300 MHz frequency range - with some observations as high as 1000 MHz.

There is one major difference between the 200 and 591 MHz stripline exposures and their effects on brain [ATP] and [CP]. Exposures at both frequencies resulted in significant decreases in [ATP] at all exposure intervals. The 591 MHz exposures resulted in significant decrease in brain [CP] exceeding the decrease in [ATP] at each exposure interval. It was noted during the experiments that approximately 1 of 5 exposed brains had normal CP levels with an accompanying greater decrease in brain [ATP] than usually seen. However, at each exposure interval, brain [CP] was significantly decreased from control levels when statistically comparing the group with controls by a 2 group comparison Student's t-test. The 200 MHz microwaves caused significant decrease in brain [ATP] at all exposure intervals, but no exposure brain [CP] group was statistically different than controls. For all time intervals all but one of 31 [CP] observations for 200 MHz microwaves fell within the 95% confidence limit for controls.

We hypothesize on the basis of finding decreased brain [ATP] but normal [CP] that 200 MHz microwaves inhibit the CP - CP-kinase - ADP interactions such that the system is incapable of transferring the high energy phosphate bond from CP to ADP to yield creatine plus ATP. (Under normal conditions the CP to ADP transferase action (CP-kinase) to yield creatine and ATP occurs so rapidly that the brain [CP] can drop as low as 60% of normal and still sustain [ATP] within normal limits.) These data support the concept that the microwave exposures at 200 MHz and 591 MHz CW, 13.8 mW/cm^2 surface power density, inhibit mitochondrial electron transport chain function.

We compared our findings at 591 MHz, CW with the horn antenna to those in a microstrip system. The SAR derived from the horn antenna was 3.39 times the value for the same exposure in the microstrip system as shown below:

Exposure 591 MHz, CW	SAR (mW/g) P_i = 1 mW/cm	SAR (mW/g) P_i = 10 mW/cm	SAR (mW/g) P_i = 20 mW/cm
Microstrip System	0.185	1.85	3.70
Horn Antenna	0.62	6.2	12.4

When using the horn antenna (Figure 7), the maximum change in brain NADH fluorescence was observed at 5.9 mW/cm^2, or 3.2 W/kg. In the microstrip system the maximum value was reached between 10 and 15 mW/cm^2 (approximately 2.33 W/kg). The difference between the two systems may be due to: 1) the electric and magnetic fields in the horn system oriented 90° with respect to the electric and magnetic fields (respectively) in the microstrip system and there may be a field orientation effect, and/or 2) there being a difference in the uniformity of the electrical field pattern between the two systems, and/or 3) errors in measuring the SAR for the two systems.

When the 591 MHz, 13.8 mW/cm^2 CW microwave exposures in the microstrip system were increased to 10, 15 and 20 min, the NADH fluorescence essentially stabilized by 10-12 min in all animals. The change in brain NADH fluorescence, [ATP] and [CP] for all exposures through 20 min are shown in Figure 13. The NADH fluorescence curve correlates well with the changes observed in brain [ATP] and [CP]. The data indicate that the prolonged exposures resulted in increased brain NADH fluorescence which should, and did, correlate with decreased [ATP] with increasing exposure interval. The data suggest that from 5 to 20 min there were increased numbers of mitochondrial electron transport chains being inhibited as reflected in the increased NADH fluorescence and decreased [ATP] and [CP]. Further, the apparent ability of compensatory action by the mitochondria between 0.5 and 3 min was exceeded by the microwave inhibition of function from 3 through 20 min where brain NADH fluorescence increased followed by decreasing [ATP] and [CP]. During the 5 to 20 min exposures, brain [ATP] and [CP] decreased to as low as 60% and 46% of controls respectively. The increase in brain [CP] between 10 and 20 min exposures (when [ATP] continues to decrease) suggest that CP-kinase activity was decreasing and could not keep up with the normal interaction of CP + CP-kinase + ADP$\longrightarrow$ATP + creatine requirements for maintaining brain [ATP]. Additional work is required to clarify this point.

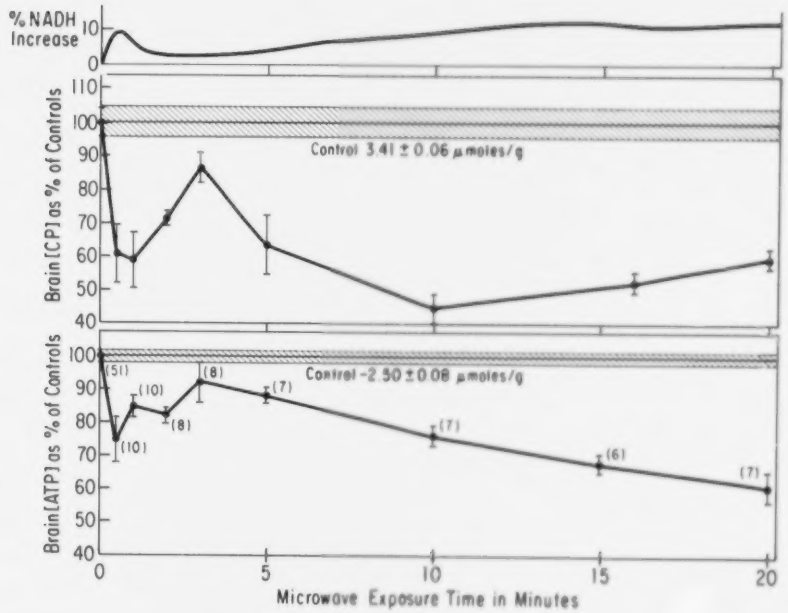


Figure 13. Rat brain NADH fluorescence, [CP] and [ATP] vs time of 591 MHz, CW, 13.8 mW/cm^2 surface power density exposure (Mean ± SEM).

STUDY 3. THE EFFECT OF SINUSOIDAL AMPLITUDE MODULATION AND PULSED MICROWAVE RADIATION ON BRAIN ENERGY METABOLISM

Amplitude Modulation

The effect of sinusoidally modulating the amplitude of the 591 MHz microwaves delivered (4, 8, 12, 16, 20, 24, 28 and 32 Hz) to rat brain was studied by NADH fluorescence changes in animals exposed to surface power densities of 10 mW/cm^2 and 20 mW/cm^2. The results of this study as observed in one of six animals - since each animal must serve as its own control - is shown in Figure 14. The maximum change in the NADH fluorescence was observed at modulation rates of 16-24 Hz. The CW exposures at 10 and 20 mW/cm^2 incident power density resulted in an NADH change equal to the 15 Hz modulation exposures. The change in NADH fluorescence at 4, 8, 12, 28 and 32 Hz modulation frequency was 82.6, 84.4, 92.7 and 82.6% of maximum, respectively. Thus amplitude modulation (sinusoidal) preferential frequency response was observed from 16-24 Hz, which was 22.2% higher than the 4 and 32 Hz modulated microwaves. This exceeds the normal level of fluctuation in the NADH fluorescence - recording system and is considered significant.

Pulsed Microwaves

The results of 591 MHz, 5 μsec/pulse, 500 or 250 pulses/sec, are shown in Figure 15. The results of 4 different animals are shown for the 500 pps rates, and only 1 animal for the 250 pps rate. The maximum and minimum observed changes of NADH fluorescence are shown in addition to 2 intermediate curves for the 500 pps modulation rate. The threshold in all instances fell between 0.4-0.5 mW/cm^2 at 500 pps rate. (The solid triangle and open circle curves had NADH fluorescence changes of 0.3% at 0.6 W/kg SAR, essentially the same as 0.33% for the solid circle curve - and were not shown in Figure 15 to avoid confusion.) In contrast, when the pulse rate was dropped to 250 pps, the 5 μsec/pulses, 591 MHz exposures had a threshold of 1.8-1.9 mW/cm^2. These data suggest that a change in duty cycle (e.g., from 0.0025 to 0.00125) may have a nonlinear relationship between threshold surface power density for a change in fluorescence of rat brain and the duty cycle, e.g., cutting the pulse length by half resulted in a 4-fold increase in threshold surface power density required to cause a change in NADH fluorescence. Additional work is necessary to confirm this suggestion.

ATP and CP assays were performed on animals exposed to 591 MHz, 13.8 mW/cm^2 surface power density at three modulation conditions: (1) CW, (2) 16 Hz amplitude modulated (sinusoidally), (3) 5 μsec/pulse at 500 pps microwave exposures for 0.5, 1 and 5 min exposures. These data are shown in Table 7. Our results show that the exposures (CW or 16 Hz amplitude modulation or 5 μsec/pulses at 500 pps) that caused an increase in brain NADH fluorescence at three exposure intervals also resulted in statistically significant decreases in brain [ATP] and [CP] for the three intervals. The results at 16-24 Hz sine wave amplitude modulation and the 5 μsec, 500 pps were essentially the same as CW as observed in the rate and magnitude of shift in the NADH fluorescence.

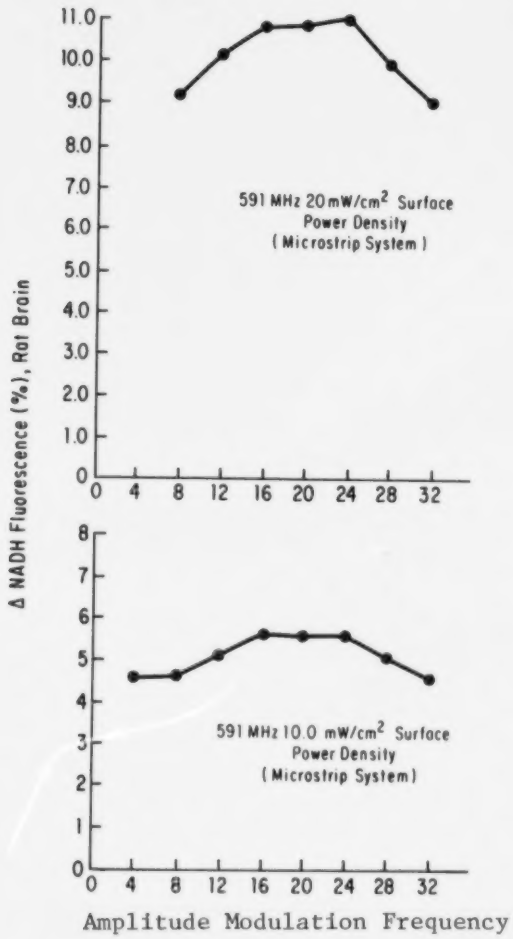


Figure 14. Amplitude modulation (sinusoid) frequency effects on rat brain NADH from 591 MHz, 10 or 20 mW/cm^2.

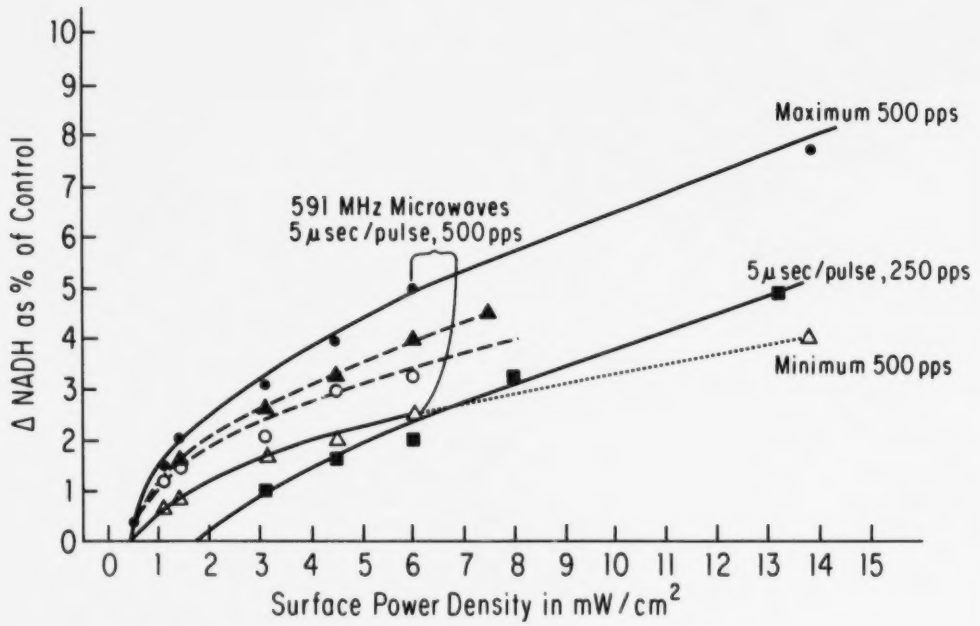

Δ NADH as % of Control
Surface Power Density in mW/cm²
Maximum 500 pps
591 MHz Microwaves
5 μsec/pulse, 500 pps
5 μsec/pulse, 250 pps
Minimum 500 pps

Figure 15. Effect of surface power density on rat brain NADH from 591 MHz, 5 μsec/pulse, 500 pps and 250 pps square wave pulsed modulated exposures.

TABLE 7. THE EFFECTS OF MODULATION ON ATP AND CP CONCENTRATION USING 591 MHz AND 13.8 mW/cm^2

Exposure:	Continuous Wave			16 Hz Amplitude Modulation			500 pps, 5 μsec/pulse		
	ATP	CP	n	ATP	CP	n	ATP	CP	n
	% of control	% of control		% of control	% of control		% of control	% of control	
Sham	100 ± 1.2	100 ± 2.36	51	100 ± 1.2	100 ± 2.6	51	100 ± 1.2	100 ± 2.6	51
0.5 min	74.8 ± 6.6	60.6 ± 8.7	10	72.1 ± 4.9	53.8 ± 7.2	6	79.3 ± 4.9	68.1 ± 5.21	6
1 min	84.9 ± 3.4	58.9 ± 8.6	10	83.3 ± 4.0	57.1 ± 8.7	6	86.8 ± 4.6	63.3 ± 6.00	6
5 min	88.8 ± 1.9	63.6 ± 8.9	8	82.7 ± 5.1	59.9 ± 6.7	6	88.1 ± 2.3	60.3 ± 7.50	6

	ATP μmoles/g	CP μmoles/g	n
Sham (control)	2.50 ± 0.03	3.41 ± 0.09	51

Mean ± SEM; P = probability of being different from control by unpaired Student's t-test, $P<0.01$ unless shown

Individual animal results are shown in Appendix II.

General Conclusions

The brain NADH fluorescence changes observed at 200 and 591 MHz exposures were similar in shape. The increase in NADH fluorescence began immediately after the initiation of the microwave exposure, reached a maximum at 0.5 min, decreased between 0.5 and 3 min, and slowly increased through 5 min for the 591 MHz. The 200 MHz exposure resulted in immediate increase at the beginning of the exposure, reached a maximum at 0.5 min, returned toward control level through approximately 1.0 to 1.25 min, and slowly increased through 5 min. The threshold level for increasing NADH fluorescence was observed to be 0.007 and 0.047 W/kg for the 200 and 591 MHz exposures respectively. There may be specific frequency difference in the 200 MHz vs the 591 MHz exposures in affecting brain NADH fluorescence. From NADH fluorescence electrical field strength data (Figure 11) at 200 and 591 MHz exposures it is possible that E field (V/m) is the major factor in the observed changes and may be observed at other frequencies.

The 200 and 591 MHz, 13.8 mW/cm^2, CW exposures resulted in significant decreases in brain [ATP] at both frequencies. The 591 MHz exposures caused significant decreases in brain [CP] which were always greater than the brain [ATP] decrease for each exposure interval. In contrast, the 200 MHz exposures caused no significant decrease in brain [CP] for 0.5, 1.0, 2.0, 3.0 and 5 min exposures. In normal brain cell physiology, [CP] decreases approximately 40% prior to observing any decrease in [ATP] due to the reaction of CP + CP-kinase + ADP $\longrightarrow$ ATP + creatine being so rapid in sustaining ATP levels in the brain. Thus it is concluded that there is a frequency specific effect at 200 MHz in inhibiting CP-kinase such that CP can not be utilized in supporting the brain [ATP].

The 2450 MHz, 13.8 mW/cm^2, CW exposures resulted in no significant changes in brain NADH fluorescence, [CP] and [ATP]. Since the SAR at 2450 MHz is twice that of the SAR for 591 MHz and eight times the SAR for 200 MHz in the microstrip, it must be concluded that the 2450 MHz exposures from 0.5 to 5 min had no effect on brain NADH fluorescence, [CP] and [ATP].

From these data it is concluded that there are frequency specific effects as observed by the differences in 200, 591 and 2450 MHz, CW exposures of rat brain. The 200 and 591 MHz exposures interfere with ATP synthesis as evidenced by significant decreases in brain [ATP] and increased NADH fluorescence at both frequencies. It is concluded that 200 MHz and 591 MHz exposures inhibit the mitochondrial electron transport chain function. The inhibition is hypothesized to be microwave induced molecular oscillation, at some location(s) along the chain which, due to rigid stereospecificity requirements for normal function, inhibits the flow down the chain. This results in increased brain NADH fluorescence and decreased [ATP]. At the 591 MHz exposures brain [CP] was decreased greater than [ATP] at all exposure intervals. (This is expected from the normal CP- CP-kinase function of rapidly rephosphorylating ADP to ATP in maintaining [ATP] at normal levels.) However, the 200 MHz exposures did not cause decreased [CP] at any interval even though brain [ATP] was significantly decreased. It is concluded that 200 MHz, 13.8 mW/cm^2,

CW exposures inhibited CP-kinase activity such that it could not function to sustain brain [ATP]. The 2450 MHz, 13.8 mW/cm^2,CW exposures did not affect energy metabolism as indicated by no significant difference between exposed and control NADH fluorescence levels, [ATP] and [CP].

The brain NADH fluorescence levels at the 200 MHz, CW and the 591 MHz, CW and pulsed, gave a similar pattern as a function of surface power density (mW/cm^2). When looking at ΔNADH fluorescence levels as a function of SAR (W/kg) for 200 and 591 MHz exposures, it is obvious that there is a distinct difference in the threshold SAR, and the magnitude of ΔNADH increase for a given SAR. When looking at ΔNADH fluorescence as a function of the E-field the threshold (V/m) is essentially the same for the 200 and 591 MHz exposures.

The increase in brain NADH fluorescence, decrease in [ATP] (200 and 591 MHz exposures), and decrease in [CP] (591 MHz exposures) were observed at exposures which did not raise the brain temperature in our exposure situation. It is possible that [ATP] and [CP] decreases could be due to focal point heating which does not result in an increase in tissue temperature. However, the probability of increased brain NADH fluorescence, accompanied by decreased [ATP] and [CP] when no temperature increase was observed in the brain for the power levels used (13.8 mW/cm^2) makes it probable that these changes are not due to increased temperature effects.

The hypothesized effect of microwave inhibition of electron transport chain function due to inducing dipole oscillations and subsequent inhibition due to stereospecificity requirements of the molecule for the chain to function is essentially the same type of mechanism as proposed for microwave inhibition of CP-kinase. Similarly, any enzyme-substrate combinations might be affected by microwaves in the same manner. There are multiple enzymes (reductases, dehydrogenases and transferases) associated with entry points to, along and at the end of the electron transport chain. These types of changes have not been seen in vitro by other authors but virtually all these experiments have been done at frequencies above 1 GHz [21-23] which is consistent with the lack of observed effects noted in this study at 2450 MHz. It is possible that there are multiple locations (preceeding, along and terminating the chain) where microwave induced dipole (thus molecular) oscillations could occur. Each such location could have a different natural resonance frequency, threshold E-field, and E-field to produce maximum effect, requirements dependent upon molecular structure, critical role of the molecule (including metal atoms where involved) in its specific function at the location of activity. Thus, there may be multiple frequency specific sites (enzymes, cofactors, cytochromes, etc.) and different threshold E-field values where microwaves could inhibit electron transport chain function. Similarly there may be multiple anabolic and catabolic enzyme reactions throughout the cell which could be microwave inhibited at the specific frequency(ies) and E-field level(s) that may be below thermogenesis. Each enzyme system would have to be studied individually to ascertain such effects. The NADH fluorescence methods for following the function of the electron transport chain only shows that there has been an effect at some point along the chain. It is recognized that

there are multiple enzyme focal points along the respiratory chain of which only one would have to be inhibited to produce the observed effects of increased brain NADH fluorescence and decreased [ATP] and [CP]. It is essential, however, that the frequencies from 1 to 800 MHz be scanned to determine if there are narrow or broad frequency specific effects on such enzyme(s) function(s).

Since energy metabolism is a common denominator essential to the life of all cells and tissue types, such studies should be pursued, in detail, in critical tissues and across the frequency spectrum where microwave induced protein oscillations would be anticipated and previously observed (1 to 800 MHz) [19].

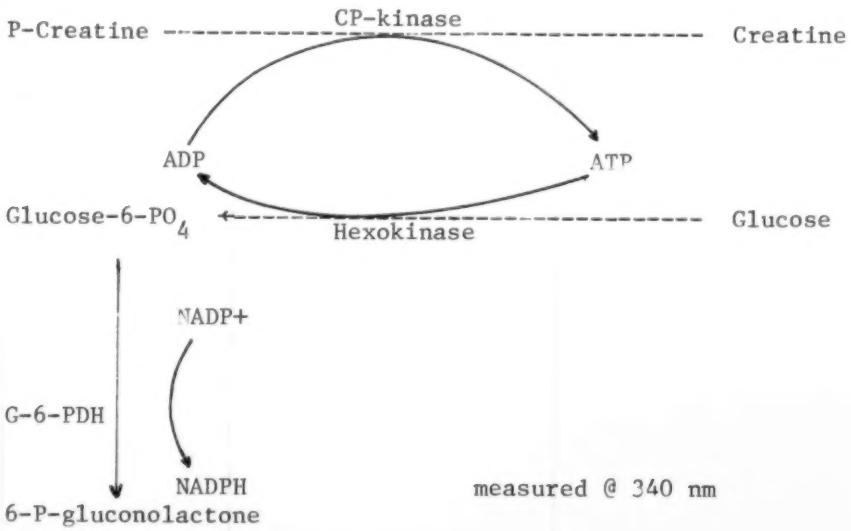
APPENDIX I

ATP AND CP ASSAY METHOD

ASSAY: ATP and P-Creatine (Gilford Spectrophotometer)

REFERENCE: O. H. Lowry and J. V. Passonneau, "A Flexible System of Enzymatic Analysis", Academic Press, New York, 1972.

REACTIONS AND THEORY:



These reactions will go essentially to completion because the equilibrium of the glucose-6-PO_4 dehydrogenase is greatly in favor of 6-P-gluconolactone formation.

REAGENTS AND APPARATUS:

A. Tissue extraction reagents

1. 3 N perchloric acid (25.7 ml of 70% PCA diluted to 100 ml with H_2O store @ 5°C
2. 0.004 M EGTA (1.52g/1) slowly pH to 7.0 with KOH
3. Neutralizing solution
   2 M KOH (11.2g (100) + 0.5 M KCL (3.73 g/100) +
   0.5 M Triethanolamine-HCL (9.3g/100)
   Store @ 5°C and prepare every two weeks.

4. -10°C Bath
5. Sorvall Centrifuge
6. Tissue pulverizer
7. Liquid nitrogen

B. Assay Reagents:

1. Assay Buffer
   0.25 M Tris-HCL (3.95g/1)
   0.25 M Tris-Base (3.03g/1)
   0.001 M $MgCl_2$ (0.203g/1)
   0.0005 M Dithiothreitol (0.080g/1)

   No need to pH-this combination has a pH of 8.1.
   Stable at RT for many weeks.

2. NADP+ (Sigma N-1630) 40 mg/ml H_2O
   Store @ -15°C
3. 0.01 M ATP (Sigma A-3127) 6 mg/ml H_2O
   Store @ -15°C
4. 0.01 M P-Creatine (Sigma P-6502) 4 mg/ml H_2O
   Store @ -15°C
5. 0.1 M Glucose 1.8 g /100 ml H_2O
   Store @ -15°C
6. ADP (Sigma A-0127) 20 mg/ml H_2O
   Store @ -15°C
7. Glucose-6-PO DH (Sigma G-6378) 28 U/ml

   (4 mg/30 ml 0.05 M Tris + .0005 (DTT) Dithiothreitol)
   Store @ -15°C
8. Hexokinase (Sigma H-4502) 112 U/ml
   (4 mg/12 ml 0.05 Tris + .0005 (DTT) Dithiothreitol)
   Store @ -15°C
9. Creatine Phosphokinase (Sigma C-3755) 720 U/ml 0.05 Tris + .0005 (DTT) Dithiothreitol

   (4 mg/0.9 ml 0.05 Tris + .0005 (DTT) Dithiothreitol)
   Store @ -15°C
10. Gilford spectrophotometer

Tissue Extraction Technique:

1. Pipet 0.75 ml of 3 N PCA into a nalgene centrifuge tube. Place this tube into a liquid nitrogen precooled Dewar flask containing a metal centrifuge sleeve and enough 95% ethanol to cover at least 1/2 of this sleeve.
2. Place the sample ().15 - .30 g) directly onto the frozen PCA. After samples have been weighed, immediately pipet an additional 0.75 ml of PCA into each tube.
3. Stopper the tube to prevent H_2O condensation.
4. Transfer the nalgene tubes with stoppers to the -10°C bath for 25 min. Periodically gently agitate these tubes to assure good mixing.

5. Transfer the tubes to an ice bath and pipet 5 ml of 0.0004 M EGTA into each tube. With a glass stirring rod gently mix the samples 3 or 4 times during the 20 min. incubation.
6. Centrifuge the samples at 14,500 rpm in the Sorvall centrifuge for 10 minutes.
7. Remove a volume in excess of 4 ml from each tube.
8. Pipet exactly 4.0 ml into a second nalgene centrifuge tube.
9. Pipet exactly 1.8 ml of the neutralizing solution into the 4.0 ml PCA extract. The procedure should be done quickly and with the aid of a Vortex mixer. Return this neutralized tube to the ice bath for about 5-10 minutes.
10. Remove the perchlorate by centrifuging for 5 min at 10,000 rpm in the Sorvall centrifuge.
11. Remove at least 1 ml of supernate for assay. (Keep in ice bath until assayed.)

COMMENTS: Perchloric acid will inhibit the assay enzymes if not completely removed. Myokinase contamination is greatest source of error in this assay and great care must be taken to be sure no small fragments of tissue are pipetted into the tube to be neutralized.

ASSAY SYSTEM:

Place in cuvette:

0.75 - 1.0 ml Assay Buffer
0.005 - 0.25 ml Sample
0.1 ml NADP+
0.01 ml Glucose-6-phosphate dehydrogenase

The contents of the cuvette are mixed by shaking and placed in a spectrophotometer. Record the initial O.D. after waiting 2-4 minutes to allow any G-6-PO_4 to react.

Now add 0.01 ml hexokinase
0.01 ml Glucose

Mix and replace into spectrophotometer and follow the reaction with the recorder until no change in optical density is observed. (4-6 min) Record the final O.D.

The ΔO.D. determination at this point is the μm of ATP in the cuvette.

Now add 0.005 ml ADP
0.01 ml CP-kinase

Mix and replace into spectrophotometer and follow the reaction on the recorder until no further increase in optical density is observed.

Record the final O.D.

The ΔO.D. determination at this point is the number of micromoles (μm) of creatine phosphate (CP) in the cuvette.

Calculations:

$$\mu m \text{ ATP in cuvette} = \frac{\text{Total volume of cuvette}}{6.27} \times \text{O.D.}_{ATP}$$

$$\Delta\text{O.D.}_{ATP} = \text{Final O.D.}_{ATP} - \text{Initial O.D.}$$

There is no reagent blank correction in the ATP determination.

$$\mu m \text{ CP in cuvette} = \frac{\text{Total vol. cuvette}}{6.27} \times \Delta\text{O.D.}_{CP}$$

$$\Delta\text{O.D.}_{CP} = \text{Final O.D.}_{CP} - \text{Final O.D.}_{ATP} - \Delta\text{O.D.}_{RB}$$

A reagent blank correction must be made in the calculation of CP since the ADP added is slightly contaminated with ATP.

All samples and the reagent blank should be done in duplicate.

Periodically, standards should be done to see if the rates of the reactions have changed. An easy way to do this is to simply add 5µℓ of 0.01 M ATP to a cuvette that ATP and CP have already been determined. When this reaction is completed, add 5µℓ of 0.01 M CP and follow on the recorder (chart speed set on slow).

Calculation of g - wet wt in the cuvette is as follows:

$$\frac{\text{grams wet wt.}}{6.5 + 0.8 \text{ (grams wt wt.)}} \times \frac{4}{5.8} = \text{g/ml of PCA extract}$$

i.e., if use 250 µℓ in assay then

(g/ml) x (0.25 ml) = grams in cuvette

therefore:

$$\text{orginal tissue m/g} = \frac{\mu m \text{ in cuvette}}{\text{g in cuvette}}$$

Modifications need to Assay ATP & CP at 3 ml vol. in Beckman DB spectrophotometer.

The tissue extraction is identical as that stated above.

Reagents changes:

1. Assay Buffer - same as above
2. NADP+ - 150 mg/ml H_2O
3. 0.03 M ATP - 18 mg/ml H_2O
4. 0.03 M CP - 12 mg/ml H_2O
5. 0.4 M Glucose - 7.2 g/100 ml H_2O
6. ADP - 50 mg/ml H_2O

7. Glucose-6-PO_4DH - same as above
8. Hexokinase - same as above
9. Creatine phosphokinase - same as above

Assay Changes - Place in cuvette

2.0 ml Assay Buffer
1.0 ml PCA extract
0.01 ml NADP+
0.02 ml G-6-PO_4DH

Mix by inversion, place in spectrophotometer and record initial O.D.

Then add:

0.02 ml HK
0.01 ml Glucose

Mix by inversion, place in spectrophotometer and record final O.D. after optical density ceases to increase.

Total vol. in cuvette for ATP determination is 3.06 ml.

Then add:
0.005 ml ADP

Mix by inversion and record final O.D. after no increse in O.D. is observed in approximately 5 minutes.

To determine creatine phosphate add:

0.02 ml CP-kinase

Mix by inversion and record final O.D. after no increase in optical density is observed. Approximately 20 minutes.

Total vol. in cuvette for CP determination is 3.085 ml

Calculation:

6.27 extinction coefficient for NADH

ATP

$$\mu m/g = \Delta O.D.\left(\frac{\text{Vol. in cuvette ml}}{6.27}\right) \div \frac{\text{sample wt. g}}{(6.50 + 0.8\ \text{wt.})\ 5.8/4}$$

$$= \frac{(\text{vol. ml})\ (6.5 + 0.8\ \text{wt})\ (1.45)}{6.27 \times \text{sample wt. g}} \times \Delta O.D.$$

(ex) $\frac{(3.06) \times (1.45)\ (6.5 + 0.8\ wt)}{6.27\ wt} \times \Delta O.D. =$

$\Delta O.D. \frac{(28.8405 + 3.5496\ wt)}{6.27} = \frac{(4.5998 + 0.5661\ wt) \times \Delta O.D.}{wt}$

$= \frac{(4.5998}{wt.\ g} + 0.5661) \times \Delta O.D. = \mu m\ ATP/g$

CP

$\frac{3.085 \times 1.45\ (6.5 \times 0.8\ wt)}{6.27 \times wt.} = \frac{(29.076 + 3.5786\ wt)}{6.27 \times wt}$

$= \frac{(4.6373 + 0.5707\ wt)}{wt}$ $\qquad$ $\frac{(4.6373}{wt.\ g} + 0.5707) \times \Delta O.D. = \mu m\ CP/g$

APPENDIX II
DATA FROM INDIVIDUAL ANIMALS BY EXPERIMENTS

Sodium Pentobarbital Anesthesia, 35.6°C Brain, Controls

ATP	CP	n
2.50 ± 0.03 μmoles/g	3.41 ± 0.09 μmoles/g	51

Urethane Anesthesia, 35.6°C Brain, Controls

Rat No.	ATP μmoles/g	CP μmoles/g
1	2.53	4.01
2	2.51	3.51
3	2.52	3.42
4	2.29	3.20
5	2.46	3.70
6	2.50	3.61
7	2.35	2.72
8	2.45	3.30
9	2.64	3.50
10	2.40	3.38
	n = 10	n = 10
	$\bar{X}$ = 2.47	$\bar{X}$ = 3.44
	σ = 0.10	σ = 0.34

Urethane Anesthesia, 37°C Brain

Rat No.	ATP µmoles/g	CP µmoles/g
1	2.21	2.68
2	2.40	2.95
3	2.18	2.54
4	2.29	2.63
5	2.30	2.75
6	2.49	2.60
7	2.48	2.95
8	2.38	2.83
9	2.37	2.89
10	2.41	2.62
	n = 10	n = 10
	$\bar{x}$ = 2.35	$\bar{x}$ = 2.74
	σ = 0.10	σ = 0.15

Urethane Anesthesia, 39°C Brain

Rat No.	ATP µmoles/g	CP µmoles/g
1	2.01	2.63
2	1.87	2.02
3	2.37	2.29
4	2.48	2.02
5	2.16	2.58
6	2.04	2.27
7	2.09	2.10
8	1.99	2.05
9	2.18	2.34
10	2.42	2.42
11	2.60	2.89
12	2.39	2.43
13	2.40	2.99
14	2.41	2.87
15	2.06	2.44
	n = 15	n = 15
	$\bar{x}$ = 2.23	$\bar{x}$ = 2.43
	σ = 0.22	σ = 0.31

Urethane Anesthesia, 41°C Brain

Rat No.	ATP µmoles/g	CP µmoles/g
1	1.87	2.10
2	1.74	1.86
3	1.82	1.37
4	1.77	1.07
5	1.89	1.62
6	1.64	1.09
7	1.90	1.55
8	1.60	1.40
9	1.85	1.42
10	1.66	1.55
	n = 10	n = 10
	$\bar{x}$ = 1.77	$\bar{x}$ = 1.50
	σ = 0.1	σ = 0.32

Urethane Anesthesia; Brain Taken to 41°C, Allowed to Cool to 37°C

Rat No.	ATP µmoles/g	CP µmoles/g
1	2.01	2.55
2	1.83	2.67
3	2.04	3.26
4	2.12	2.63
5	2.37	2.77
6	1.79	2.05
	n = 6	n = 6
	$\bar{x}$ = 2.03	$\bar{x}$ = 2.66
	σ = 0.21	σ = 0.39

Urethane Anesthesia; 35.6°C Brain: 591 MHz, 13.8 mW/cm^2, CW Microwave Exposures

Rat No.	0.5 min ATP µmoles/g	0.5 min CP µmoles/g	No.	1 min ATP µmoles/g	1 min CP µmoles/g	No.	3 min ATP µmoles/g	3 min CP µmoles/g	No.	5 min ATP µmoles/g	5 min CP µmoles/g
1	2.00	1.93	1	2.07	1.84	1	2.18	3.14	1	2.29	2.04
2	1.69	1.89	2	2.04	1.95	2	2.35	2.99	2	2.20	2.50
3	1.81	2.11	3	2.16	1.94	3	2.30	2.71	3	2.11	2.11
4	1.90	1.98	4	2.21	1.87	4	2.23	2.89	4	2.13	1.87
5	1.74	2.09	5	1.98	1.89	5	2.31	2.81	5	2.15	2.03
6	1.80	2.03	6	2.10	2.02	6	2.33	2.85	6	2.21	2.09
	n = 6	n = 6		n = 6	n = 6		n = 6	n = 6		n = 6	n = 6
	$\bar{x}$ = 1.82	$\bar{x}$ = 2.01		$\bar{x}$ = 2.09	$\bar{x}$ = 1.92		$\bar{x}$ = 2.28	$\bar{x}$ = 2.90		$\bar{x}$ = 2.18	$\bar{x}$ = 2.11
	σ = 0.11	σ = 0.09		σ = 0.08	σ = 0.07		σ = 0.07	σ = 0.15		σ = 0.07	σ = 0.21

URETHANE ANESTHESIA, 35.6°C BRAIN TEMPERATURE

200 MHz, CW, 13.8 mW/cm^2 Microwave Exposures

	ATP		CP	
Controls	2.50 ± 0.03 µmoles/g	51	3.41 ± 0.09 µmoles/g	51

0.5 min			1 min			2 min			3 min			5 min		
Rat No.	µmoles/g ATP	CP	Rat No.	µmoles/g ATP	CP	Rat No.	µmoles/g ATP	CP	Rat No.	µmoles/g ATP	CP	Rat No.	µmoles/g ATP	CP
1	2.04	3.47	1	2.41	2.85	1	2.05	3.47	1	1.85	3.75	1	2.12	2.41
2	2.21	3.35	2	2.30	3.65	2	2.40	3.43	2	1.66	3.37	2	2.02	3.88
3	1.82	3.57	3	2.26	3.10	3	2.33	3.62	3	1.89	3.36	3	2.12	3.57
4	1.80	3.27	4	2.31	3.48	4	2.30	3.74	4	1.99	3.29	4	2.52	3.22
5	2.11	3.40	5	2.34	3.56	5	1.90	3.54	5	2.28	3.36	5	2.18	3.49
6	2.43	3.39	6	2.05	3.15	6	2.18	3.68	6	2.68	3.40	6	2.10	3.76
7	2.13	3.30												
n = 7		7	n = 6		6	n = 6		6	n = 6		6	n = 6		6
$\bar{X}$ =	2.08	3.99	$\bar{X}$ =	2.28	3.30	$\bar{X}$ =	2.19	3.58	$\bar{X}$ =	2.23	3.42	$\bar{X}$ =	2.18	3.39
σ =	0.22	0.102	σ =	0.14	0.31	σ =	0.19	0.12	σ =	0.38	0.16	σ =	0.18	0.50

Control ATP 95% Confidence Limit
= 2.22 to 2.78 moles/g
Exposed - 11 of 31 individually within - 95% C.L.

Control CP 95% Confidence Limit
= 2.58 to 4.24 moles/g
Exposed - 30 of 31 within 95% C.L.

URETHANE ANESTHESIA, 35.6°C BRAIN

2450 MHz, 13.8 mW/cm^2 S.P.D., CW Microwave Exposures

Controls			0.5 Min Exposure			1 Min Exposure		
Rat no.	ATP µmoles/g	CP µmoles/g	Rat No.	ATP µmoles/g	CP µmoles/g	Rat No.	ATP µmoles/g	CP µmoles/g
1	2.59	3.52	1	2.44	3.80	1	2.20	3.47
2	2.42	4.49	2	2.61	4.24	2	2.59	4.26
3	2.58	4.52	3	2.70	4.46	3	2.28	3.52
4	2.37	3.43	4	2.40	3.53	4	2.65	4.29
5	2.64	4.56	5	2.68	4.29	5	2.21	3.48
6	2.66	4.41	6	2.51	3.49	6	2.51	4.16
7	2.49	4.49	7	2.65	4.21	7	2.23	4.19
8	2.37	3.51	8	2.48	3.43	8	2.21	3.49
9	2.40	3.46		n = 8	n = 8		n = 8	n = 8
10	2.32	3.37		$\bar{x}$ = 2.56	$\bar{x}$ = 3.93		$\bar{x}$ = 2.36	$\bar{x}$ = 3.86
11	2.57	4.63		σ = 0.12	σ = 0.42		σ = 0.19	σ = 0.40
12	2.71	4.58						
13	2.52	3.49						
14	2.44	4.57						
15	2.47	3.45						
	n = 15	n = 15						
	$\bar{x}$ = 2.50	$\bar{x}$ = 4.03						
	σ = 0.12	σ = 0.55						

URETHANE ANESTHESIA, 35.6°C BRAIN

2450 MHz, 13.8 mW/cm^2 S.P.D., CW Microwave Exposures

Rat No.	2 Min Exposure ATP µmoles/g	2 Min Exposure CP µmoles/g	No.	3.0 Min Exposure ATP µmoles/g	3.0 Min Exposure CP µmoles/g	No.	5 Min Exposure ATP µmoles/g	5 Min Exposure CP µmoles/g
1	2.42	4.15	1	2.27	4.10	1	2.43	4.04
2	2.29	3.60	2	2.30	3.58	2	2.59	4.44
3	2.33	3.53	3	2.45	4.25	3	2.61	4.47
4	2.44	4.08	4	2.31	4.19	4	2.40	3.69
5	2.31	3.59	5	2.45	3.60	5	2.47	3.75
6	2.50	4.18	6	2.47	3.54	6	2.45	3.72
7	2.45	4.14	7	2.41	3.82	7	2.56	3.70
8	2.32	3.55	8	2.40	4.21	8	2.58	3.80
	n = 8	n = 8		n = 8	n = 8		n = 8	n = 8
	$\bar{x}$ = 2.38	$\bar{x}$ = 3.85		$\bar{x}$ = 2.38	$\bar{x}$ = 3.91		$\bar{x}$ = 2.51	$\bar{x}$ = 3.95
	σ = 0.08	σ = 0.31		σ = 0.08	σ = 0.31		σ = 0.08	σ = 0.33

591 MHz, CW, 13.8 mW/cm , 35.6°C Brain Temperature

	10 min µmoles/g		15 min µmoles/g		20 min µmoles/g	
	ATP	CP	ATP	CP	ATP	CP
1	1.99	1.80	1.62	1.76	1.36	1.80
2	1.82	1.49	1.97	1.93	1.51	2.04
3	1.74	1.50	1.92	1.75	1.73	2.32
4	1.80	1.42	1.49	1.63	1.72	2.27
5	1.99	1.71	1.61	1.89	1.42	1.96
6	2.02	1.47	1.67	1.81	1.59	2.01
7	2.20	1.60			1.46	1.99
	n = 7	n = 7	n = 6	n = 6	n = 7	n = 7
	$\bar{x}$ = 1.91	$\bar{x}$ = 1.57	$\bar{x}$ = 1.71	$\bar{x}$ = 1.80	$\bar{x}$ = 1.54	$\bar{x}$ = 2.04
	σ = 0.21	σ = 0.14	σ = 0.19	σ = 0.11	σ = 0.14	σ = 0.16

Control	ATP	CP	n
Mean ± SEM	2.50 ± 0.03 µmoles/g	3.41 ± 0.09 µmoles/g	51

Urethane Anesthesia, 35.6°C Brain; 16Hz, A.M., 591 MHz, 13.8 mW/cm^2 Microwaves

	ATP µmoles/g	CP µmoles/g	n
Control	2.50 ± 0.03* Mean ± SEM	2.41 ± 0.09*	51

Rat No.	0.5 Min Exposure	
1	2.13	2.50
2	1.49	1.32
3	1.91	2.09
4	1.68	1.29
5	2.05	2.34
6	1.51	1.41
	n = 6	n = 6
	$\bar{x}$ = 1.80	$\bar{x}$ = 1.83
	σ = 0.27	σ = 0.55

Rat No.	1 Min Exposure	
1	2.23	2.54
2	2.18	1.50
3	1.80	1.32
4	1.74	1.24
5	2.26	2.39
6	2.29	2.69
	n = 6	n = 6
	$\bar{x}$ = 2.08	$\bar{x}$ = 1.95
	σ = 0.25	σ = 0.65

Rat No.	5 Min Exposure	
1	1.65	1.35
2	2.27	2.45
3	2.20	2.40
4	1.77	1.44
5	2.31	2.21
6	2.23	2.38
	n = 6	n = 6
	$\bar{x}$ = 2.07	$\bar{x}$ = 2.04
	σ = 0.29	σ = 0.51

Urethane Anesthesia, 35.6°C Brain;
5 μsec/pulse, 500 pps, 591 MHz, 13.8 mW/cm^2 Microwaves

Rat No.	ATP μmoles/g	CP μmoles/g	Rat No.	ATP μmoles/g	CP μmoles/g	Rat No.	ATP μmoles/g	CP μmoles/g
1	1.72	1.98	1	2.24	2.31	1	2.29	2.12
2	2.25	2.24	2	2.37	3.16	2	2.07	2.16
3	2.21	2.76	3	1.84	2.26	3	2.10	2.08
4	1.83	2.03	4	2.38	3.20	4	2.34	1.98
5	2.16	2.84	5	1.85	2.28	5	2.09	2.02
6	1.70	2.08	6	2.35	2.41	6	2.32	2.10
	n = 6	n = 6		n = 6	n = 6		n = 6	n = 6
	$\bar{x}$ = 1.98	$\bar{x}$ = 2.32		$\bar{x}$ = 2.17	$\bar{x}$ = 2.60		$\bar{x}$ = 2.20	$\bar{x}$ = 2.07
	σ = 0.26	σ = 0.38		σ = 0.26	σ = 0.45		σ = 0.13	σ = 0.07

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